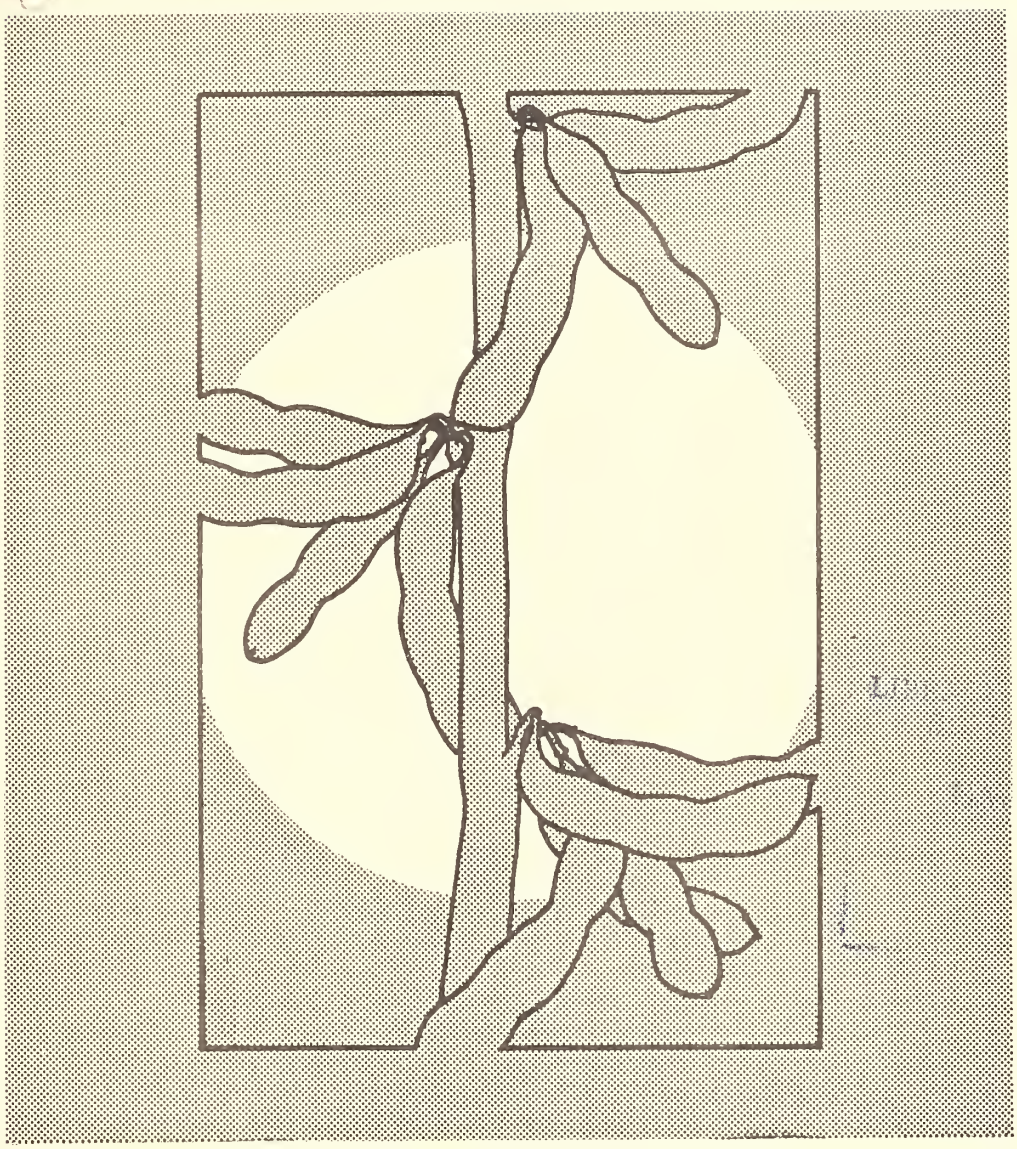
Soybean Genetics Newsletter



Volume 4 April 1977

Agricultural Research Service - USDA
and Department of Agronomy
Iowa State University
Ames, Iowa 50011

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I. FOREWORD

Through the generosity of the National Soybean Processors Association, Volumes 1 and 2 of the Soybean Genetics Newsletter were offered to soybean scientists throughout the world free of charge. The USDA-ARS provided financial support for Volume 3 and continues their support for Volume 4. In an effort to keep expenses at a minimum, however, we have included on our mailing list only those scientists who have specifically requested the Newsletter. Last fall we included, with our request for research notes, a coupon to be returned, placing the respondent's name on the mailing list for Volumes 4, 5 and 6. The response has been gratifying.

Anyone interested in genetics and breeding of soybeans and closely-allied genera, or related biochemistry, entomology, pathology, physiology, taxonomy, etc., will receive the Soybean Genetics Newsletter upon request.

Of special note in this issue is an article, starting on page 23, by H. H. Hyland, titled "Procedures for handling soybean germplasm between the United States and foreign countries."

Again this year, the production of the Soybean Genetics Newsletter is a group effort. Technicians Carol Winger and Holly Heer, secretary Linda Martin, and graduate students Steve Broich and David Stelly have served.

Reid G. Palmer

II. ANNOUNCEMENT

The International Working Group on Soybean Rust has announced the inception of a newsletter, Soybean Rust News, with the first issue to be published this spring.

INTERNATIONAL WORKING GROUP ON SOYBEAN RUST (IWGSR)

Soybean is one of the important food, feed and oil crops in the world today. The full potential of its production capacity in the tropics is yet to be exploited. Among the various problems confronting the farmers, diseases rank high.

In the tropics and subtropics, soybean rust (Phakopsora pachyrhizi) is one of the important diseases limiting soybean production. There is mounting evidence that soybean rust can reduce yield as much as 60%.

Research on the pathogen, its distribution, its epidemiology, and its destructiveness and on sources of resistance and methods of control are urgently needed to provide the understanding required to suppress or prevent losses caused by the disease.

Even though the disease is now widely prevalent only in the tropics, its potential to gain entry into the major soybean production areas in temperate zones cannot be underestimated.

Realizing the importance of the rust problem and recognizing the values to be gained by cooperative efforts, workers actively engaged in soybean rust research formally organized the "International Working Group on Soybean Rust" (IWGSR) during the Regional Soybean Conference jointly organized by INTSOY, AVRDC, and the Government of Thailand and held in Chiang Mai, Thailand, 23-27 Feb. 1976. Dr. C. Y. Yang of AVRDC was elected chairman, Dr. K. R. Bromfield of USDA, ARS, vice chairman and Mr. S. Shanmugasundaram of AVRDC, secretary of this group.

The objectives of this group are:

1) To mount and intensify a consolidated research program to combat soybean rust disease.
2) To enroll as participating members scientists from all over the world who are interested in and working on soybean rust.

3) To hold at intervals workshops, symposia, or conferences which will bring scientists together to exchange ideas and thoughts on the soybean rust problem.
4) To publish once a year relevant information and results of soybean rust research stemming from workers in various countries.
5) To distribute for evaluation germplasm and breeding lines having rust resistance.
6) To serve as an agency for the rapid exchange of germplasm and information.

REQUEST FOR CONTRIBUTIONS TO THE FIRST ISSUE OF "SOYBEAN RUST NEWS"

Research articles, reports, notes, announcement of resistant or tolerant germplasm, and any other news item related to soybean rust are requested, and they will be accepted until April 1, 1977. Address all correspondence regarding the SRN to:

S. Shanmugasundaram
Soybean Coordinator
Soybean Rust News
AVRDC, P.O. Box 42
Shanhua, Tainan 741
Taiwan, R.O.C.

RULES FOR CONTRIBUTORS

1) Information in the SRN will be informal to stimulate the exchange of ideas and information among soybean rust scientists. SRN articles may be preliminary in nature and speculative in content, and should not be regarded as equivalent to papers published in formal scientific journals. Even so, such reports can be very valuable and helpful, if viewed in the proper perspective. Data presented in the SRN are not to be used in other publications without the consent of the respective authors.
2) Contributions should be in English, typed double spaced and not more than four 8½" by 11" pages. You may send as many separate contributions as you wish. Send two copies for each article.
3) Correspondence regarding an article should be on a separate page.
4) Photographs should be glossy black/white prints of high quality with good dark and light contrasts. Drawings for graphs and charts should be

prepared with India ink on good quality tracing paper. Typewritten matter is not usually acceptable on graphs and charts. A good size for photographs is 5" by 7" and drawings is what will fit on an 8½" by 11" page.

5) Except for possible minor editing, manuscripts will be published as received from contributors.

6) Title your report, place your name(s), name of university, institution or company under the title. Please give complete address. [For contributors outside Taiwan (R.O.C.): please send reports by airmail.]

7) Citations of recent publications are most welcome.

International Working Group on Soybean Rust (IWGSR)

Membership Form

Name: _____

Position: _____

Mailing Address: _____

_____

_____

Qualifications: _____

_____

_____

_____

Major area of Research Interest: _____

_____

_____

_____

_____

Minor area: _____

_____

_____

Any Publications on Soybean Rust You Have Authored (Please enclose a reprint for each): _____

_____

_____

_____

_____
Signature

Return this form to:

Mr. S. Shanmugasundaram
Secretary, (IWGSR)
Crop Coordinator (Soybean), AVRDC
P.O. Box 42, Shanhua, Tainan 741
Taiwan, Republic of China

P.S. Would appreciate your returning the Membership Form on or before July 31, 1977.

III. REPORT OF SOYBEAN GENETICS COMMITTEE

A) The current members of this committee and the expiration dates of their terms are:

R. L. Bernard, USDA (1978)
Davenport Hall
University of Illinois
Urbana, IL 61801

H. R. Boerma (1980)
Dept. of Agronomy
University of Georgia
Athens, GA 30602

R. I. Buzzell (1979)
Agr. Canada, Res. Station
Harrow, Ontario, NOR 1GO
Canada

H. H. Hadley, Chm. (1979)
Dept. of Agronomy
University of Illinois
Urbana, IL 61801

E. E. Hartwig, USDA (1978)
Delta Branch Exp. Station
Soybean Prod. Res.
Stoneville, MS 38776

T. C. Kilen, USDA (1980)
Delta Branch Exp. Station
Soybean Prod. Res.
Stoneville, MS 38776

R. G. Palmer, USDA
(Editor of Soybean Genetics Newsletter)
Agronomy Department
Iowa State University
Ames, IA 50011

B) Organization of the Committee:

1) The Committee will be composed of six elected members and the editor of the Soybean Genetics Newsletter.

2) The term of the elected members will be three years. After a member has been off for one year, he (she) can be reelected. The Committee will elect two new members each year; a simple majority is needed for election. The members will be elected prior to February 1 of each year, by a mail ballot conducted by the chairman.

3) At the annual meeting of the Committee (usually in February in conjunction with the Soybean Breeding and Genetics Workshop), the two new members and the two retiring members of the Committee are eligible to attend and vote.

4) The Chairman will be elected at the annual Committee meeting and serve through the next annual meeting, and may be reelected.

C) The duties of this Committee were reviewed and revised at St. Louis, MO, February 21, 1977, and the following procedures were established:

1) Maintain Genetic Collection.

The Genetic Collection is divided into four categories:

a) Type Collection includes all published genes of soybeans, preferably in the original strains (excluding U.S. and Canadian name varieties, which are maintained in a separate collection) plus certain mutants or strains that appear to the Committee to have potential genetic interest.

b) Isoline Collection includes adapted varieties Clark, Harosoy and Lee, into which have been backcrossed single genes or combinations of genes. Also included are certain genes or combinations with Chippewa, Wayne and Williams.

c) Linkage Collection includes linkage combinations and the various genetic recombinations.

d) Cytological Collection includes translocations, inversions, deficiencies, trisomics, tetraploids, etc.

Collections a, b and c are maintained at Urbana, Illinois, with R. L. Bernard as curator. Collection d is maintained at Ames, Iowa, with R. G. Palmer as curator.

D) Manuscript review and genetic symbol approval.

The Soybean Genetics Committee requests that researchers submit all manuscripts concerning qualitative genetic interpretation and symbols to the Committee Chairman. This review by the Genetics Committee will serve to insure orderly identification and use of genetic nomenclature and to avoid conflict of symbols. This will also allow assignment of type collection designations (T-numbers) prior to publication, so that these T-numbers may be used in the journal article to identify parental lines.

E) Soybean Genetics Newsletter notes.

All notes for the Newsletter should be sent to the SGN editor, R. G. Palmer, who will ask the Soybean Genetics Committee to review those articles concerning qualitative genetic interpretation and symbols. Genetic symbols reported in the Newsletter will have the same status as those published in scientific journals.

Rules for Genetic Symbols

I) Gene Symbols

a) A gene symbol shall consist of a base of one to three letters, to which may be appended subscripts and/or superscripts as described below.

b) Genes that are allelic shall be symbolized with the same base letter(s) so that each gene locus will be designated by a characteristic symbol base.

c) The first pair of genes reported for a gene locus shall be differentiated by capitalizing the first letter of the symbol for the dominant or partially dominant allele. (Example: Ab, ab. Ab is allelic and dominant to ab.) If genes are equivalent, codominant, or if dominance is not consistent, the capitalized symbol may be assigned at the author's discretion.

d) When more than two alleles exist for a locus, the additional alleles or those symbolized subsequently to the pair first published shall be differentiated by adding one or two uncapitalized letters or a number as a superscript to the base. (Example: R, r^m, r.) This shall be the only use of superscripts. The base for the additional alleles is capitalized only when the gene is dominant or equivalent to the allele originally designated with a capitalized symbol. The superscript may be an abbreviation of a descriptive term. When allelism is discovered for a gene previously assigned a symbol, the previous symbol may be used as the superscript.

e) Gene pairs with the same or similar effects (including duplicate, complementary, or polymeric genes) should be designated with the same letter base differentiated by numerical subscripts, assigning 1, 2, 3, 4, etc., consecutively in the order of publication. (Example: The y series for chlorophyll deficiency.) This shall be the only use of subscripts. Letter subscripts should not be used. The subscript 1 is automatically a part of the first reported gene symbol for each base but may be omitted until the second symbol is assigned.

f) Base letters may be chosen so as to indicate apparent relationships among traits by using common initial letters for all loci in a related

group of traits. Examples are P for pubescence type, R for disease reaction (plus two initials of the pathogen to complete the base), and L for leaf shape.

g) The distinction between traits that are to be symbolized with identical, similar, or with unrelated base letters is necessarily not clear cut. The decision for intermediate cases is at the discretion of the author but should be in accordance with previous practices for the particular type of trait. The following sections concern supplementary symbols that may be used whenever desired as aids to presentation of genetic formulas.

h) A dash may be used in place of a gene symbol to represent any allele at the indicated locus. The locus represented should be apparent from its position in the formula. (Example: A_ represents both AA and Aa.)

i) A question mark may be used in place of a symbol when the gene is unknown or doubtful, or it may be used as a superscript to the base symbol for the same purpose. (Example: $a^{?}$ indicates that the latter is an unknown allele at the A locus.)

j) Plus symbols may be used in place of the assigned gene symbols of a designated standard homozygous strain when this will facilitate presenting genetic formulas. The standard strain may be any strain selected by the worker, as long as the strain being used and its genetic formula are made explicit.

II) Linkage and Chromosome Symbols

a) Linkage groups and the corresponding chromosomes shall be designated with Arabic numerals. Linkage shall be indicated in a genetic formula by preceding the linked genes with the linkage group number and listing the gene symbols in the order that they occur on the chromosome.

b) Permanent symbols for chromosomal aberrations shall include a symbol denoting the type of aberration plus the chromosome number(s) involved. Specific aberrations involving the same chromosome(s) shall be differentiated by a letter as follows: The symbol Tran shall denote translocations. Tran 1-2a would represent the first case of reciprocal translocations between chromosomes 1 and 2, Tran 1-2b the second, etc.

The symbol Def shall denote deficiencies, Inv inversions, and Tri primary trisomics. The first published deficiency in chromosome 1 shall be symbolized as Def 1a, the second as Def 1b, etc. The first published inversion in chromosome 1 shall be denoted as Inv 1a, etc. The first published primary trisomic shall be designated with the Arabic numeral that corresponds to its respective linkage group number.

c) Temporary symbols for chromosomal aberrations are necessary, as it may be many years before they are located on their respective chromosomes. Tran 1 would represent the first case of a published reciprocal translocation; Tran 2, the second case, etc. The first published deficiency shall be symbolized as Def A, the second as Def B, etc. The first published inversion shall be symbolized as Inv A, the second as Inv B, etc. The first published primary trisomic shall be designated as Tri A, the second as Tri B, etc. When appropriate genetic and/or cytological evidence is available, the temporary symbols should be replaced with permanent symbols, with the approval of the Soybean Genetics Committee.

III) Cytoplasmic Factor Symbols

a) Cytoplasmic factors shall be designated with one or more letters prefixed by cyt-. (Example: cyt-G indicates the cytoplasmic factor for maternal green cotyledons, cyt-Y indicates that for maternal yellow cotyledons.)

IV) Priority and Validity of Symbols

a) A symbol shall be considered valid only when published in a recognized scientific journal, or when reported in the Soybean Genetics Newsletter, with conclusions adequately supported by data which establish the existence of the entity being symbolized. Publication should include an adequate description of the phenotype in biological terminology, including quantitative measurements wherever pertinent.

b) In cases where different symbols have been assigned to the same factor, the symbol first published should be the accepted symbol, unless the original interpretation is shown to be incorrect, the symbol is not in accordance with these rules, or additional evidence shows that a change is necessary.

V) Rule Changes

a) These rules may be revised or amended by a majority vote of the Soybean Genetics Committee.

IV. RESEARCH NOTES

AGRICULTURE CANADA
Research Station
Harrow, Ontario

1) Soybean linkage tests.

F_2 linkage results are presented in Table 1 with a = XY, b = Xy, c = xY, and d = xy for the gene pairs listed in the form of Xx and Yy. Percentage recombination was obtained as previously (Buzzell, 1974).

The fg_1 gene appears to be loosely linked with dt_1 in Linkage Group 5. Scoring for dt_1 was done on F_2 plants and F_3 progenies; for the origin of OX250, see Buzzell (1975).

An estimate of the linkage between Fg_3 and T was obtained, but additional work is needed to map Fg_3 and Fg_4 in Linkage Group 1. Since 'Kingwa' carries td (Bernard, 1975), T and t were scored on the basis of the presence or absence of quercetin glycosides in the leaves (Buttery and Buzzell, 1973).

Table 1

Soybean F_2 linkage tests

Genes	a	b	c	d	Sum	%R	SE	Phase
OX250 (fg_1 dt_1) X OX922 (Fg_1 Dt_1)								
Fg_1 fg_1 Dt_1 dt_1	135	31	33	16	215	39.8	3.0	c
Blackhawk (fg_2 Fg_3 t ep i^i) X Kingwa (Fg_2 fg_3 T Ep i)								
Fg_2 fg_2 Fg_3 fg_3	122	42	37	18	219	54.9	3.2	r
Fg_2 fg_2 T t	126	38	44	11	219	52.6	3.5	c
Fg_2 fg_2 Ep ep	117	45	40	13	215	52.3	5.3	c
Fg_2 fg_2 i^i i	126	36	39	14	215	46.8	4.9	c
Fg_3 fg_3 T t	111	48	59	1	219	13.7	6.6	r

References

Bernard, R. L. 1975. The inheritance of near-gray pubescence color. Soybean Genet. Newsl. 2: 31-33.

Buttery, B. R. and R. I. Buzzell. 1973. Varietal difference in leaf flavonoids of soybeans. Crop Sci. 13: 103-106.

Buzzell, R. I. 1974. Soybean linkage tests. Soybean Genet. Newsl. 1: 11-14.
Buzzell, R. I. 1975. Soybean linkage tests. Soybean Genet. Newsl. 2: 10-11.

R. I. Buzzell

ASIAN VEGETABLE RESEARCH AND DEVELOPMENT CENTER
P.O. Box 42, Shanhua, Tainan
Taiwan 741, Republic of China

1) Decapitation technique to screen for photoperiod insensitivity in soybean, Glycine max (L.) Merrill.

The lack of a suitable screening technique for photoperiod response has prevented the identification of photoperiod-insensitive (P_I) genotypes. In an F_2 population, each plant has a specific genotypic constitution. To determine the photoperiod response, each F_2 plant should be subjected to at least two different photoperiods. In rice, this was accomplished by separating the component tillers of each plant and subjecting them to different photoperiods (Chandraratna, 1955).

To obtain more than one individual of the same F_2 genotype early in the growth stage, the following three different methods were tried: (a) rooting of cuttings, (b) grafting to a common stock, and (c) 'decapitation'. To test the above techniques, two strains identified as photoperiod insensitive, PI 194.647 (Acc. 215) and PI 248.407 (Acc. 1322) and one photoperiod-sensitive (PS) strain, Acc. 2120 (a pureline from PI 86.736) were used.

Rooting of cuttings: Ten plants from each strain were grown under long days, and cuttings of equal length were taken from each plant and rooted. The objective was to obtain two or more individuals for each F_2 genotype for testing under different photoperiods. This technique did not work satisfactorily because the time taken for rooting varied with variety, and the cuttings were not satisfactorily rooted.

Grafting: Ten plants from each strain were grown as above. In this method, the cuttings were grafted to a common stock plant instead of rooting. The plants used as stock were grown to the first unifoliate leaf stage and cut between the unifoliate and cotyledonary node. The test cuttings (scions) were wedge grafted. The scion and stock were held together by a plastic clip. The graft union time between plants within and between varieties varied considerably. Therefore, this technique was also not satisfactory.

Decapitation: Garner and Allard (1925) found a number of plant species whose plant parts, when subjected only to critical day length or lower, produced flowers, whereas those parts of the same plant exposed to longer than critical photoperiod did not. The localized induction reaction was proved by Borthwick and Parker (1938) in their experiment with two-branched plants obtained by decapitating the young plants. We used a similar technique to identify the photoperiod response of a genotype.

When the individual plants of the 3 strains reached the first unifoliate leaf stage (fully open), we decapitated the meristem. The buds in the axils of the unifoliate leaf were stimulated and produced two axillary branches. The axillary buds at the cotyledonary node normally failed to grow but were removed if they did grow. When the first trifoliate leaf appeared on each branch, the treatments began. From the beginning, the plants were exposed to the sunlight for 10 hr, and then one branch was covered with black plastic and the other was left uncovered. The plants were then moved into an artificial lighted room to provide expanded photoperiod (sunlight + artificial light = 16 hr) using fluorescent and incandescent lamps (about 580 ft-c.). Thus one branch was continuously under 10-hr photoperiod while the other was in a 16-hr photoperiod. To compare this technique with the conventional technique of screening the whole plant, a set of plants of each strain were grown under the two photoperiod regimes.

The results of 3 identical trials are presented in Table 1. Using the decapitation technique, we can distinguish between PS and P_I strains and can use this technique to study the inheritance of photoperiod sensitivity.

References

Borthwick, H. A. and M. W. Parker. 1938. Photoperiodic perception in Biloxi soybeans. Bot. Gaz. 100: 374-387.

Chandraratna, M. F. 1955. Genetics of photoperiod sensitivity in rice. J. Gen. 53: 215-223.

Garner, W. W. and H. A. Allard. 1925. Localization of the response in plants to relative lengths of day and night. J. Agric. Res. 31: 555-565.

Table 1

Days to flowering in decapitation technique trials with two photoperiods and three varieties

	AVRDC Acc. no.	Days to flower: Decapitation technique 10 hr	Decapitation technique 16 hr	No decapitation 10 hr	No decapitation 16 hr
Trial I	215	29 ± 0.0	29 ± 0.0	30 ± 2.0	30 ± 2.4
	1322	27 ± 2.2	28 ± 2.0	28 ± 2.0	30 ± 1.9
	2120	44 ± 3.0	-	45 ± 2.4	-
Trial II	215	33 ± 3.0	34 ± 2.5	35 ± 1.7	36 ± 1.2
	1322	37 ± 1.9	38 ± 2.1	38 ± 1.9	38 ± 2.0
	2120	46 ± 2.7	-	46 ± 2.3	-
Trial III	215	49 ± 1.0	49 ± 1.0	44 ± 2.0	42 ± 3.0
	1322	46 ± 0.8	46 ± 0.9	44 ± 1.0	42 ± 1.3
	2120	50 ± 2.4	-	48 ± 2.7	-

Date planted: Trial I Aug. 9, 1976
Trial II Oct. 6, 1976
Trial III Nov. 8, 1976

10 plants

- did not flower until 150 days after emergence when the experiment was terminated.

S. Shanmugasundaram
C. C. Wang

2) Inheritance of photoperiod insensitivity to flowering in Glycine max (L.) Merrill.

At the Asian Vegetable Research and Development Center (AVRDC) one of the objectives of our soybean program is to identify photoperiod insensitivity (P_I) in the germplasm and to develop high-yielding, widely-adapted types using P_I. Photoperiod insensitivity has been reported in soybean by Yoshida (1952), Pohjakallio and Antila (1957), Criswell and Hume (1972), and Shanmugasundaram et al. (1974). However, due to unavailability of a suitable practical technique to screen the F_2 and backcross individuals, the inheritance of P_I could not be studied accurately.

With the help of the "decapitation technique" (Shanmugasundaram and Wang,

1977) the inheritance of P_I to flowering is reported in this paper. Criswell and Hume (1972) and AVRDC (1975) reported PI 194.647 (Acc. 215) to be P_I for first flower anthesis. An Acc. 2120 (a pureline selection from PI 86.736) with high yield was sensitive to photoperiod.

Acc. 215 was crossed with Acc. 2120. The parents, F_1, and F_2 were planted and at the unifoliate leaf stage were subjected to "decapitation technique" which induced the axillary buds to produce two branches at the unifoliate leaf node. One branch was subjected to 10-hr photoperiod and the other was subjected to 16-hr photoperiod. The number of P_1, P_2, F_1, and F_2 plants screened and their photoperiod reactions are presented in Table 1. The F_1 flowered in both 10-hr and 16-hr photoperiods. But in the 16-hr photoperiod, the F_1 was delayed 35 days for flowering. Previously we established that a delay of 0 to 5 days between 10 hr and 16 hr was within two standard deviations from the mean number of days to flowering and, thus, insensitive to photoperiod. Since the F_1 exceeded the 5-day difference, it is considered to be photoperiod sensitive (PS). The PS:P_I ratio of the F_2 plants fit the expected

Table 1

Inheritance of photoperiod insensitivity in soybean

Source	No. of plants		Days to flower		Delay
	10 hr	16 hr	10 hr	16 hr	in 16 hr
P_1(215)	10	10	29	29	0
P_2(2120)	10	10	54	150+	96+
50106 F_1(215 x 2120)	6	6	40	75	35
50106 x F_2	200	200			
			Range	Range	Range
Total survived	169	169	32-57	30-90+	0-80+

Source	Photoperiod sensitive	Photoperiod insensitive
F_2 segregation		(Difference between 10 hr and 16 hr is 0-5 days)
Observed	135	34
Expected (3:1 ratio)	126.75	42.25
χ^2 =	2.148	
P =	0.25 - 0.1	

3:1 segregation ratio even though the chi-square P is slightly lower.

A few of the P_I (homozygous recessive) F_2 individuals were tested for photoperiod reaction in the F_3, using the same "decapitation technique". All were insensitive to photoperiod. Segregation in the F_2 indicates the possibility of obtaining F_2 plants which have longer days to flowering with photoperiod insensitivity (Table 2).

Further studies with other crosses are in progress to establish the inheritance of photoperiod insensitivity to flowering and its association with other characters.

Table 2
Recombination of photoperiod insensitivity
with longer days to flowering

Source	Days to first flower		Photoperiod sensitivity
	10 hr	16 hr	
P_1 215	29	29	-
P_2 2120	54	150+	+
F_1 50106(215 x 2120)	40	75	+
F_2			
50106-8	42	42	-
50106-13	33	33	-
50106-47	36	36	-
50106-91	47	47	-
50106-120	43	43	-
50106-130	47	47	-

+ = Photoperiod sensitive; - = Photoperiod insensitive.

References

Asian Vegetable Research and Development Center. 1975. Annual report for 1974. Shanhua, Taiwan, Republic of China.

Criswell, J. G. and D. J. Hume. 1972. Variation in sensitivity to photoperiod among early maturing soybean strains. Crop Sci. 12: 657-660.

Pohjakallio, O. and S. Antila. 1957. Photoperiodic characters of some bred strains of soybeans. Maataloustiet Aikak. 29: 113-120. (Field Crop Abstr. 11: 186. 1958).

Polson, D. E. 1972. Day-neutrality in soybeans. Crop Sci. 12: 773-776.

Shanmugasundaram, S. and C. C. Wang. 1977. Decapitation technique to screen for photoperiod insensitivity in soybean. Soybean Genet. Newsl. 4: 13-15.

Shanmugasundaram, S., T. S. Toung, T. D. Liou and H. B. F. Wu. 1974. Photoperiod insensitivity in soybean. Agron. Abstr. (Chicago, IL) p. 78.

Yoshida, S. 1952. Photoperiodic responses in soybean plants under long day conditions with supplemental illumination of different intensities at night (preliminary report). Proc. Crop Sci. Soc. Jpn. 21: 127-128.

S. Shanmugasundaram

BELTSVILLE AGRICULTURAL RESEARCH CENTER
Cell Culture and Nitrogen Fixation Laboratory
Beltsville, MD 20705

1) A technique for evaluating nodulation response of soybean genotypes with specific Rhizobium strains.

Previous research on the interactions of Rhizobium strains with host cultivars has involved the testing of small numbers of plants with a specific strain of Rhizobium in Leonard jar assemblies (Leonard, 1943). The Leonard jar technique in our greenhouse requires frequent watering (up to twice daily) and periodic adjustment of the wick element. We have found the traditional Leonard jar assembly technique inadequate to efficiently accommodate the large plant populations required in plant selection and genetic studies of allelism and linkage.

To efficiently accommodate large plant populations we have devised an apparatus designed to minimize care and environmental variation. The apparatus was also designed to facilitate controlled infection by specific Rhizobium strains without contamination.

The apparatus consists of two elements: (1) plant growth trays suspended above and (2) a nutrient reservoir. The plant growth trays were constructed of ¼" Plexiglas cut and formed into two boxes, 22⅞" wide, 25" long, and 6" deep. The trays are filled 5½" deep with a culture medium of sterile perlite or vermiculite. In our experience, both media have proved satisfactory for soybean plant growth and have the advantage of lighter weight than the sand traditionally used. We have observed that nodule formation on soybeans was greater with vermiculite than with sand. The floor of each box was fitted with 6 tubes 2½" long, 1¾" diameter. The tubes were placed in two rows of three 5½" apart. The rows were placed at 7" and 19" in the width of

the plant growth tray. The tubes permit the liquid nutrient solution (Johnson et al., 1958) to rise by capillary action into the culture medium. The tube ends are fitted with an 18-mesh screen to retain this material. Support runners constructed of $\frac{5}{8}$" Plexiglas glued on the sides of the planter trays suspend the trays over the nutrient solution.

The reservoir or bottom container is constructed of black $\frac{1}{2}$" Plexiglas cut and formed to 24" wide, 51$\frac{1}{4}$" long, and 4$\frac{1}{2}$" deep. The black Plexiglas was used to restrict light penetration and algae growth in the reservoir. A $\frac{1}{4}$" slant in the floor of the reservoir facilitates drainage of the fluid through a brass spigot at the lowest point. The nutrient reservoir holds 80 liters of nutrient solution as a nutrient source for the plants. This reservoir holds enough fluid to expand the service period to approximately 2 weeks without replenishment.

For tests of eight weeks' duration with soybeans, each growth tray readily accommodates 3 rows of 12 seeds each for a combined total of 72 plants for each assembly. Higher plant populations may be used in tests of shorter duration or in the case of species with seedlings of small size. Seed may be inoculated prior to planting, all seed in a given assembly receiving the same strain or mixture of strains.

The plant growth tray assembly is easily cleaned and disinfected with a mixture of 50% ethyl alcohol and 50% water or other disinfectant. After disinfection, tests have shown no evidence of contamination by residual bacteria. Prolonged usage of 8 weeks or more may produce slight algae growth on the surface of the plant growth trays. This growth has not interfered with test evaluation.

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1) Induced floral abnormality in soybean.

One of the M_3 progenies of Type-49 soybean irradiated with 15 kr gamma rays showed segregation for plants with abnormal flowers. The progeny consisted of 30 normal plants and 8 mutant plants, indicating a monogenic inheritance. The mutant plants set only a few pods and appeared almost sterile at maturity. The M_4 progenies derived from normal plants were again screened for genetic segregation and detailed studies on floral abnormality of mutant plants were made. Of the 28 progenies planted, 18 segregated and 10 bred true for normal plants, giving a close fit to 2:1 ratio. The total number of normal and mutant plants pooled over all segregating progenies were 762 and 245, respectively, which fitted closely to a 3:1 ratio, with no heterogeneity among the progenies. Thus, this character is under monogenic control.

Floral abnormality: The normal and mutant plants appeared identical until the onset of flowering, after which the differences began to set in. Most of the buds of the mutant plants did not open at all and those which did were only partially open. A close examination of these flowers revealed very interesting differences. These flowers had invariably more than 5 sepals, more than 5 petals (including two standard petals), less than 10 stamens and more than 1 pistil, but the total number of floral parts per flower never exceeded 21. In most of the cases, it was 21, with occasional flowers with 18-20 parts. It was observed that the reduction in the number of stamens was always equal to the increased number of sepals, petals and pistils. The extra sepals were always petaloid and these were in inner ring surrounded by 5 true sepals. Some of the petals and pistils had anthers on them. When dissected, these anthers showed normal pollen grains. The pollen grains from normal anthers were also viable and germinated in sucrose solution but the dehiscence was very poor.

These observations indicate that the mutant gene is primarily affecting stamen differentiation. Apparently the regulatory control for the differentiation of stamen primordial cells is less effective in the mutant plants, leaving some of the cells in confused state. Since the immediate neighboring cells are either for petals or pistils, some of these confused stamen primordial cells might be picking up regulatory signals from these and differentiat-

ing into petals or pistils. Increased number of petal cells in the inner ring might be causing a push-back reaction to convert some of the outer petal cells into petaloid sepals. As the mutant plants become older, the effect of this gene becomes more generalized and the whole bud develops into an undifferentiated mass.

The gene symbol "ft ft" (flower transformed) has been assigned for this character.

Reference

Meyer, V. G. 1966. Flower abnormalities. Bot. Rev. 32: 165-218.

B. B. Singh
A. N. Jha

2) Isozymic variations in black- and yellow-seeded isogenic lines of 'Bragg' soybean.

Occasional black seeds are noticed in 'Bragg' seed lots obtained from large multiplication plots. The frequency of such seeds varies from 10^{-5} to 10^{-7}, indicating that the black seeds result from spontaneous mutation. The plants grown from black seeds are identical to Bragg plants in every respect so that, without opening the pods on mature plants, it is impossible to distinguish the two types of plants. The mean agronomic performance of black- and yellow-seeded Bragg in replicated trials is given in Table 1. No significant difference was observed for any of the characters. Thus, these lines appear to be isogenic except for difference in seed coat color.

The preliminary data have indicated black seed coat to be a recessive trait and the character is under monogenic control. Apparently, the two lines differ with respect to one gene only. We wanted to check whether this would be reflected in terms of single biochemical difference also. The black and yellow seeds were kept in moist sterilized sand for germination. After 72 hours, the germinating seeds were separated into seed coat, cotyledons, and root-shoot axis. Three grams of each kind of tissue were ground in 3 ml phosphate buffer (pH 7.0). The homogenate was centrifuged and the supernatant was used for disc-electrophoresis. The electrophoresis was performed in Tris-glycine buffer at pH 8.5. After electrophoresis, the gels were stained for protein, and different enzymes.

Table 1
Agronomic characteristics of black- and yellow-seeded isolines of Bragg soybean

Character	Yellow seed	Black seed
Days to flower	45	45
Pubescence color	Tawny	Tawny
Flower color	White	White
Disease	Severe yellow mosaic	Severe yellow mosaic
Days to maturity	113	113
Plant height (cm.)	69.3	68.5
Pods per plant	70.4	64.9
Seeds per pod	2.10	2.21
100-seed weight	14.00 g	14.35 g
Yield kg/ha	1418	1666

No significant differences were noticed in the number of bands and banding patterns for proteins or enzymes between cotyledons and root-shoot axes of black and yellow seeds. However, the seed coats of the two lines differed quite markedly for proteins as well as for peroxidases, esterases, and acid and alkaline phosphatases. The number of bands and banding pattern for these enzymes are presented in Fig. 1. It is apparent from Fig. 1 that the black seed coat is completely devoid of proteins, peroxidases, acid phosphatases and alkaline phosphatases, whereas in the yellow seed coat at least 6 protein bands, 5 peroxidase isozymes, 5 acid phosphatase isozymes and 4 alkaline phosphatase isozymes could be detected. The black seed coat had a fast moving esterase in place of 3 esterase isozymes of yellow seed coat.

These observations indicate that the mutation affecting seed coat color is also checking the synthesis of a number of proteins and enzymes. Since the inheritance of this character is monogenic with a single phenotypic effect, the multidirectional differences at biochemical level indicate that this is probably a complex locus consisting of several closely-linked genes, all being affected simultaneously by a single mutation. Alternatively, this gene might be blocking the synthesis of a substrate (factor) which is common in the synthetic pathways of different proteins and enzymes. Still another possibility

is that this gene may have regulatory function in activating several genes.

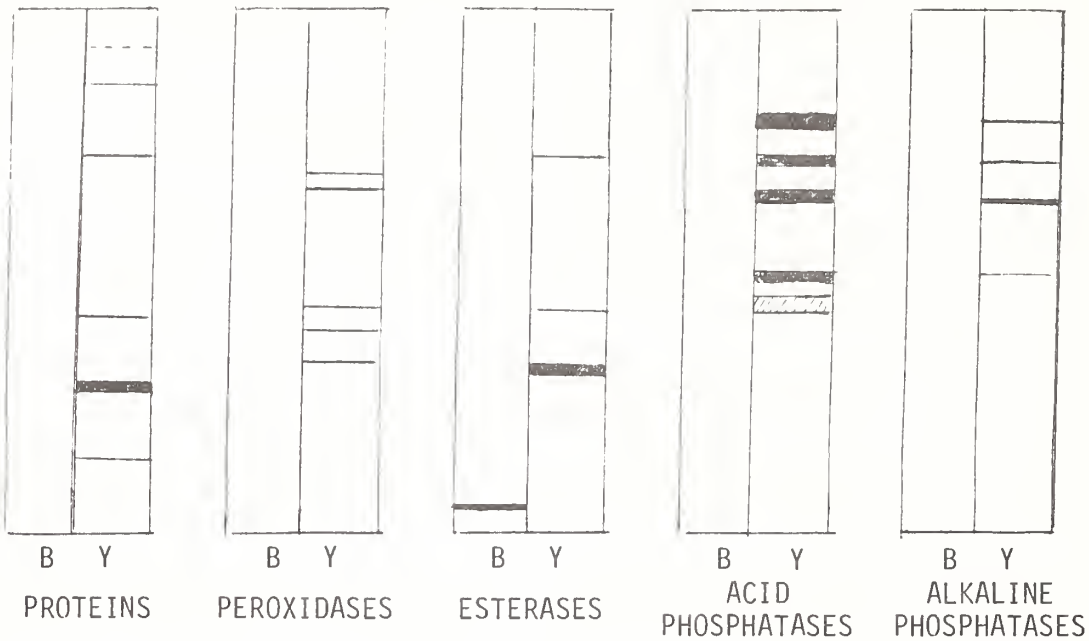

Figure 1. Isozymic variations in the seed coats of black (B) and yellow (Y) seeded isogenic lines of Bragg soybean.

S. S. Malik
B. B. Singh

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1) Procedures for handling soybean germplasm between the United States and foreign countries.

Federal, state, and commercial soybean breeders periodically receive direct requests for seed from potential foreign cooperators, including both those who are technically qualified to undertake research and those who have little experience with the crop and are initiating programs for the first time. The Germplasm Resources Laboratory in Beltsville, MD, also receives numerous requests from foreign cooperators as a result of its involvement in the international germplasm exchange program that has been operating for 25 to 30 years. In addition, U.S. breeders request specific germplasm from

foreign sources and new plant introductions are added to the soybean germplasm collections. Coordination of all these exchange activities has been the responsibility of the Plant Introduction Officer in the Germplasm Resources Laboratory in cooperation with U.S. soybean specialists.

During recent years, plant quarantine regulations in specific countries have become more strict and, in many cases, import permits are now required in advance. U.S. breeders are similarly concerned about soybeans entering our country. These facts have resulted in setting up a procedure whereby all experimental plant shipments are consigned through the following address:

Plant Germplasm Quarantine Center
Attn.: H. R. Hanes
USDA, Building 320, BARC-East
Beltsville, MD 20705

Plant inspectors of the Animal and Plant Health Inspection Service (APHIS) examine seed samples, advise whether import permits are required, and finally issue a federal phytosanitary certificate to cover the shipment in accordance with existing regulations for the receiving country. Similarly, shipments coming into the U.S. pass through the Quarantine Center where they are also inspected by APHIS prior to forwarding to the intended recipient or the curators of the soybean germplasm collection, R. L. Bernard and E. E. Hartwig.

Permanent records are kept in the Germplasm Resources Laboratory's files for all shipments passing through the Quarantine Center. Data therein are used for future reference to prevent duplication of shipments, or avoiding subsequent requests being honored until reports on prior shipments have been received. Many cases could be cited where "form" letters have gone to several potential sources of germplasm, thus resulting in useless duplication of effort. Such data is also useful in bringing together specific crop specialists within a given country who may not be aware that their colleagues have already requested or received germplasm from U.S. sources.

Whenever you receive what appears to be a form letter that may have gone to several other agencies in the U.S., you may send it to Beltsville for screening, thus preventing a certain amount of duplication of effort. Some of the most frequently requested material is maintained here at Beltsville and many additional requests are filled by the curators.

All shipments for foreign countries should be addressed to the Quarantine

Center and should include a copy of a letter of transmittal and a listing, in triplicate, of the samples enclosed. We include one copy inside the package, another comes to the Germplasm Resources Laboratory from the Quarantine Center, and the third is used by the plant inspectors. There is no need to send an advance copy to Beltsville unless some special conditions warrant advance approval. Since all exchanges are sent by air, we ask that the maximum weight be no more than three or four pounds unless some provision is worked out in advance for charges against the supplying agency.

At present, there is one exception to the above procedures, and this pertains to the "Iron Curtain" countries. We are required to write a clearance letter, in advance, through the Foreign Agriculture Service, Washington, DC, to any of these countries: Bulgaria, Czechoslovakia, East Germany, Hungary, Latvia, Lithuania, Poland, Romania, and USSR. Therefore, for these restricted countries, send a copy of the original request and the seed to:

Plant Introduction Officer
Germplasm Resources Laboratory
Building 001, BARC-West
Beltsville, MD 20705

You may transmit whatever information you wish along with any specific request for material in exchange. We will then make certain that all such information, along with your name, will get into the body of the letter. No exchanges are presently attempted with Albania, Cuba, People's Republic of China, North Korea, or North Vietnam.

We recognize there are foreign requests directed to the commercial companies the same as to our federal and state researchers. We are willing to take care of such shipments providing they are not too cumbersome and not large amounts. One additional qualification we place on handling commercial materials is whether they would expect to receive, in exchange, foreign varieties or breeding lines that would be of interest to other soybean breeders in the U.S. Some companies, as well as private breeders, may have a rather "closed" exchange, whereby they do not wish to share incoming materials. In these cases, we prefer not to become involved since all germplasm we handle is to be shared with those breeders who have an interest, and will be assigned plant introduction (P.I.) numbers. Any requests for foreign germplasm should be sent to the Plant Introduction Officer. Please include in the request as much information as possible about any reference to the material or address of

possible sources. Usually, all soybean accessions are sent first to the U.S. Regional Soybean Laboratory, Urbana, IL, or divided with that Laboratory with prior approval of the intended recipient of the seed.

We offer these services on the basis that most soybean breeders have been most cooperative in supplying us, without charge, experimental quantities required in our program of international germplasm exchange. It also permits us to maintain records in a central location covering shipments abroad. We will be glad to answer any inquiries related to these procedures.

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1) Linkage tests between Sp_1 and Ti seed proteins.*

Polyacrylamide gel electrophoresis has been used to study the Ti and Sp_1 seed proteins of the soybean. The Ti protein has been identified as the Kunitz soybean trypsin inhibitor or SBTI-A_2 (Kunitz, 1945; Rackis et al., 1962; Singh, Wilson and Hadley, 1969). The three forms of SBTI-A_2 designated as Ti^1, Ti^2 and Ti^3 are electrophoretically distinguishable from one another by their different Rf values of 0.79, 0.75 and 0.83 (Rf = mobility relative to the dye front in a 10% polyacrylamide gel anodic system using a pH 8.3 Tris-glycine buffer) respectively. The forms are controlled by a codominant multiple allelic system at a single locus (Hymowitz and Hadley, 1972; Orf and Hymowitz, 1976b). The Sp_1 protein has not been characterized. The two forms of the Sp_1 protein designated as Sp_1^a and Sp_1^b have Rf values of 0.36 and 0.42 respectively (Orf and Hymowitz, 1976a) and originally were called the "A" and "B" proteins by Larsen (1967). This protein is controlled by codominant alleles at a single locus (Larsen and Caldwell, 1968; Orf and Hymowitz, 1976a). The purpose of this study was to determine if the Ti and Sp_1 proteins were linked

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or inherited independently of each other.

The seeds used in this investigation were obtained from R. L. Bernard, USDA, Urbana. The crosses T31 (Rf 0.79 = $\underline{Ti}^1$, Rf 0.42 = $\underline{Sp_1}^b$) X 'Jefferson' (Rf 0.75 = $\underline{Ti}^2$, Rf 0.36 = $\underline{Sp_1}^a$), T31 X PI 196.172 (Rf 0.83 = $\underline{Ti}^3$, Rf 0.36 = $\underline{Sp_1}^a$) and PI 196.172 X T245 (Rf 0.75 = $\underline{Ti}^2$, Rf 0.42 = $\underline{Sp_1}^b$) were made in the field. The F_1 plants were grown in the greenhouse to produce F_2 seeds that were analyzed for segregation ratios. The whole seed procedure as outlined by Hymowitz and Hadley (1972) was used for extraction of the proteins and determination of the banding patterns. The partial seed procedure (Hymowitz and Hadley, 1972) was used to identify F_1 hybrid seed.

The distribution of the F_2 genotypes from selfed F_1 plants of each of the crosses is shown in Tables 1, 2 and 3. In each case the data show a good fit to the 1:2:1:2:4:2:1:2:1 ratio that would be expected if the two loci were independent. Therefore, we conclude that the Ti^1 locus is inherited independently of the Sp_1 locus.

Table 1

Observed and expected F_2 segregation of the Sp_1 locus and the Ti locus from selfed F_1 soybean plants of the cross T31 ($\underline{Ti}^1$, $\underline{Sp_1}^b$) X Jefferson ($\underline{Ti}^2$, $\underline{Sp_1}^a$)

Genotype	$\underline{Ti}^1$	$\underline{Ti}^1$	$\underline{Ti}^1$	$\underline{Ti}^2$	$\underline{Ti}^2$	$\underline{Ti}^2$	Total
$\underline{Sp_1}^a$ $\underline{Sp_1}^a$	14	(15)†	28	(30)	15	(15)	57 (60)
$\underline{Sp_1}^a$ $\underline{Sp_1}^b$	36	(30)	54	(60)	32	(30)	122 (120)
$\underline{Sp_1}^b$ $\underline{Sp_1}^b$	15	(15)	31	(30)	15	(15)	61 (60)
Total	65	(60)	113	(120)	62	(60)	

χ^2 = 2.17; probability = 0.98.

†expected numbers in parentheses.

Table 2

Observed and expected F_2 segregation of the Sp_1 locus and the Ti locus from selfed F_1 soybean plants of the cross T31 ($\underline{Ti}^1$, $\underline{Sp_1}^b$) X PI 196.172 ($\underline{Ti}^3$, $\underline{Sp_1}^a$)

Genotype	$\underline{Ti}^1$	$\underline{Ti}^1$	$\underline{Ti}^1$	$\underline{Ti}^3$	$\underline{Ti}^3$	$\underline{Ti}^3$	Total
$\underline{Sp_1}^a$ $\underline{Sp_1}^a$	18	(14)†	24	(28)	16	(14)	58 (56)
$\underline{Sp_1}^a$ $\underline{Sp_1}^b$	25	(28)	57	(56)	21	(28)	103 (112)
$\underline{Sp_1}^b$ $\underline{Sp_1}^b$	17	(14)	31	(28)	15	(14)	63 (56)
Total	60	(56)	112	(112)	52	(56)	

χ^2 = 5.125; probability = 0.74.

†expected numbers in parenthesis.

Table 3

Observed and expected F_2 segregation of the Sp_1 locus and the Ti locus from selfed F_1 soybean plants of the cross PI 196.172 ($\underline{Ti}^3$, $\underline{Sp_1}^a$) X T245 ($\underline{Ti}^2$, $\underline{Sp_1}^b$)

Genotype	$\underline{Ti}^2$	$\underline{Ti}^2$	$\underline{Ti}^2$	$\underline{Ti}^3$	$\underline{Ti}^3$	$\underline{Ti}^3$	Total	
$\underline{Sp_1}^a$ $\underline{Sp_1}^a$	13	(7.1875)†	15	(14.375)	7	(7.1875)	35	(28.75)
$\underline{Sp_1}^a$ $\underline{Sp_1}^b$	14	(14.375)	22	(28.75)	13	(14.375)	49	(57.5)
$\underline{Sp_1}^b$ $\underline{Sp_1}^b$	7	(7.1875)	16	(14.375)	8	(7.1875)	31	(28.75)
Total	34	(28.75)	53	(57.5)	28	(28.75)		

χ^2 = 6.739; probability = 0.57.

†expected numbers in parentheses.

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Rackis, J. J., H. A. Sasame, R. K. Mann, R. L. Anderson and A. K. Smith. 1962. Soybean trypsin inhibitors: Isolation, purification and physical properties. Arch. Biochem. Biophys. 98: 471-478.

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Theodore Hymowitz

2) Single seed selection for carbohydrate content in soybean seeds.

We have investigated the feasibility of using single seed selection to change the sugar content of soybeans. In order to study the distribution of sucrose, raffinose, stachyose, and total sugar in the embryo, seeds of the varieties 'Jogun' and 'Hokkaido' were sliced into three portions of approximate equal weights. Slices were made parallel to the root-shoot axis with Position I containing the root-shoot axis and Position III lying distal from it. Differences among positions were found for sucrose, stachyose, and total sugar. Stachyose concentration was lowest in Position II. Sucrose and total sugar were highest in Position I.

In two sets of crosses ('Bansei' X PI 81.785 [G. gracilis] and 'Sioux' X 'Altona'), variances of percent sugar of F_2 seeds borne on F_1 plants were compared with those of parental seeds in order to determine if genetic variation could be detected. Significant genetic variation was not observed (Table 1). These results indicate that sugar content in soybeans is primarily under maternal control and that selection among individual seeds on a heterozygous plant

Table 1

Variances of sugar percentages among individual seeds on parental and F_1 plants

	df	Sugar			
		Sucrose	Raffinose	Stachyose	Total
Parental (pooled)	46	0.7998	0.0855	1.2834	1.7112
F_2 (PI 81.785 X Bansei)	34	1.1312	0.0293	0.9834	1.5787
F_2 (Sioux X Altona)	56	0.4595	0.0813	0.4538	0.7868

would not be effective in changing this trait.

Analysis of F_2 seeds on F_1 plants from the cross Altona X Sioux gave mean values of 3.4% for sucrose, 1.2% for raffinose, 4.6% for stachyose, and 9.2% for total sugar. Reciprocal F_1 plants gave mean values of 3.6% for sucrose, 1.2% for raffinose, 4.8% for stachyose, and 9.6% for total sugar. The data give no indication of cytoplasmic effects on sugar content in this cross.

Sugar content was determined by GLC analysis of TMS derivatives. Because the derivatizing reagent used ("tri-Sil Z") is quite expensive, the high cost of the technique limits its usefulness in a breeding program.

S. J. Openshaw
H. H. Hadley

3) Seed responses of four soybean cultivars to microwave treatments.

In the fall of 1975, we were contacted by C. J. Brannon, Jr. of Agriculture, Inc. (Fort Lauderdale, FL) and O. S. Gray of Energy Transfer Corporation (Evansville, IN) regarding a possible boost in seed yield and number of pods in soybeans treated by microwaves. As a result, in 1976 we tested the responses of four cultivars adapted to Illinois to several microwave treatments. This is a preliminary and partial report of performances in that test.

The four cultivars were 'Amsoy 71', 'Calland', 'Wells', and 'Williams'. Seed was obtained from Illinois Foundation Seeds, Inc. and showed germination percentages of 83, 86, 85 and 91 respectively. Six lots of seed from each cultivar were sent to Energy Transfer Corporation, c/o Delta Steel Company, West Memphis, AR, for treatment, while one lot was retained at Urbana as a

"nontravelling" check. Treatments were as indicated in Tables 1 and 2. Five lots were actually treated and one became a "travelling" check.

We conducted two trials, since for some reason unknown to us one treatment was changed when applied to Williams (Tables 1 and 2). Thus three cultivars were tested in one trial, enabling us to get information on possible interaction between genotype and treatment. But the second trial only allowed us to compare a slightly different set of treatments on Williams.

Seeds from the control and treated lots were planted May 17, 1976 in 3-row plots at rates necessary to give 170 plants (based on germination behavior of untreated seed) per 5.7 m (19 ft) of row. Only the middle row was harvested, after being trimmed by 46 cm (1.5 ft) at each end. The design was a randomized complete block, split plot with three replications. Cultivars constituted main plots with treatments being randomized within each cultivar.

In the 3-cultivar test, seed yields (dry matter content) varied significantly in respect to cultivars but not to treatments (Table 1). However, it is noteworthy that a significant treatment X cultivar interaction was obtained. In Wells, all treated lots gave lower yields, but in Calland, all treated lots gave higher yields than the corresponding travelling checks. In Amsoy 71, some treated lots yielded more and others yielded less than the travelling check. Comparisons between yields of travelling and nontravelling checks showed no consistent advantage of one over the other. They doubtlessly contributed more to the interaction mean square than we would like. However, even if the checks are omitted, the rank correlations for seed yields of treated lots are quite low. Precision of the test seems adequate, since the coefficient of variability (CV) is 6.3% which is reasonably low for a seed yield performance test.

In the test involving Williams only, no significant differences were found among treatments (Table 2). In this test the CV was 8.1%.

From our data, it would seem that, on an average, microwave treatment has no effect on seed yield. Still, the indication of interaction between cultivars and treatments suggests that microwave treatment may have an effect on some cultivars in certain environments. If cultivars do respond differently, the researcher (and the farmer) should require very careful testing of specific cultivars before predicting responses to microwave treatment either for research purposes or for commercial production. Probably similar precautions should be taken for different environments.

Table 1

Seed yields of three soybean cultivars treated with microwaves
g dry matter per plot (Urbana, 1976)

Treatment milliamps/seconds	Cultivar			Mean
	Amsoy 71	Calland	Wells	
740/10†	992	898	1081	990
740/5	1084	901	1102	1029
250/10	1078	1005	1070	1051
185/40	991	958	1022	990
185/30	1131	939	1035	1035
Check (travelling)	1018	842	1166	1009
Check (nontravelling)	1098	828	1056	994
Mean	1056	910	1076	1013

†740 milliamps for 10 seconds.

Table 2

Seed weights (dry matter in g/plot) of Williams soybeans
treated with microwaves (Urbana, 1976)

Treatment milliamps/seconds	Replications			Mean
	1	2	3	
740/10†	879	927	967	924
740/5	978	942	963	961
740/8	894	1043	838	925
185/40	853	1024	863	913
185/30	949	916	1063	976
Check (travelling)	948	810	974	911
Check (nontravelling)	993	1083	1070	1049
Mean	928	964	963	951

†740 milliamps for 10 seconds.

Estimating degree of effects of microwave treatment on pod number is difficult because pod number varies so much from plant to plant unless the plants are very regularly spaced. We measured number of pods in 30 cm (1 ft) sections of rows. A section was taken from the center position in the middle row of each plot. Even with this procedure the CV was high (21.2%). In the 3-cultivar test, differences between cultivars were significant ($F = 17.6$, 2 and 4 df), but differences associated with treatments were not ($F = 1.5$, 6 and 36 df). The interaction mean square was also nonsignificant ($F = 0.63$, 12 and 36 df).

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1) Spacing and soybean breeding.

The problem of spacing is worldwide and concerns all crops. The usual plant density for soybeans is 25-65 plants/m^2 (Scott and Aldrich, 1970). Nowadays there are efforts to put more soybeans on the field. For example, Gogerty (1976) pointed out that the yield increases as the row distance narrows. Generally it has been observed that more to the north the rows come closer and the plant density increases (Anonymous, 1976). Yield tests are made generally with invariable seeding rates or even with the same number of vigorous seeds per unit, without any account to individual demand on spacing. Whigham (1975) conducted a worldwide comparison with a uniform plant density (40 plants/m^2).

There are two ways to solve the spacing problem: turn space to the requirements of the single plant or find a suitable type to the most efficient space. As shown with bush snap beans, the yield target can be reached and even beaten in the range of higher plant densities (Gretzmacher, 1974, 1975).

The spacing experiment: Fig. 1 illustrates the relationship between the reciprocal effect of spacing and yield. The broken line shows the yield per single plant for various plant densities required to get equivalent yields per area. The full line shows the yield obtained by a spacing experiment with the

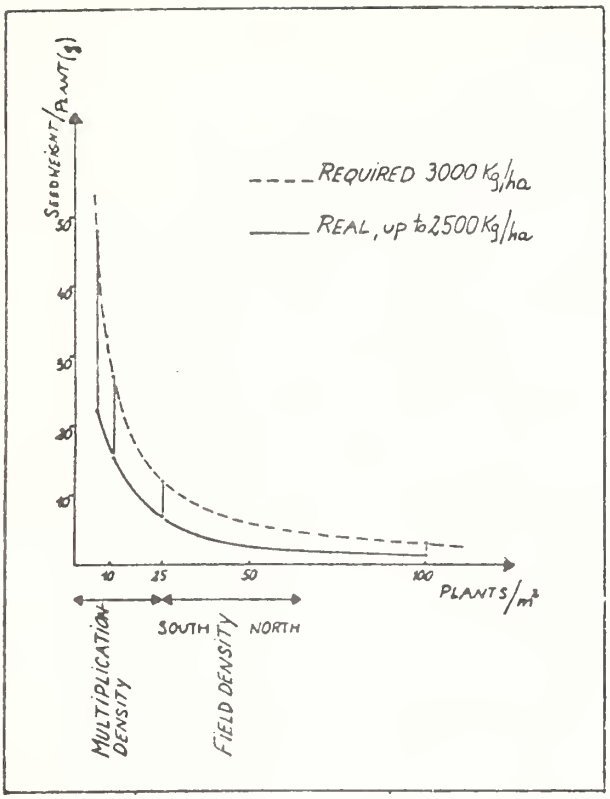

Figure 1. Required and real yield per single plant in relation with plant density

Hungarian variety 'Pannonia 10' (maturity class 0) in Vienna (48°12' north, 9.7°C and 668 mm as average) which was brought in a square disposition with 4 replications: 10 X 10, 20 X 20 (Standard), 30 X 30 and 40 X 40 cm space per single plant. This gives 100, 25, 11.1 and 6.25 plants/m^2. The resulting yield parameters are presented in Table 1.

In the first part, the data show for single plants a strong decrease with increasing plant density. Even the 100-seed weight decreases from 22.0 g (40 X 40) to 19.9 (30 X 30), 20.1 (20 X 20) and finally to 16.7 g (10 X 10). The very low ratio of seeds per pod was influenced in the same way with its optimum of 1:1.74 at 11 plants/m^2 and minimum of 1:1.62 at 100 plants/m^2. A similar distribution shows the height of the plant--70.1 cm at 11 plants/m^2 and 55.9 cm at 100 plants/m^2. The same correlation was observed for the number of branches, leaves and nodes and the diameter and the weight of the stem--a decrease by increasing plant density. Only the "pod-free-space" (harvest-criterion) increases from 5.9 cm at 11 plants/m^2 to 14.9 cm at 100 plants/m^2.

The experiment with 4 spacings has shown that the highest yield per unit gives 25 plants/m^2. The optimal total yield might be expected in the range up to 50-65 plants/m^2, as could be deduced from the statistical distribution. See also the narrowing of the two lines in Fig. 1.

Multiplication of F_1 and F_2: In the last 2 years, 30 X 30 cm has been used for the multiplication of the F_1. This is not the optimum spacing for single plant yield, but more comparable to growing characteristics than 40 X 40 cm; therefore it gives also an impression of the growing type. This is

Table 1

Yield parameters of 4 different spacings

Plants/m^2	Number of pods			Number of seeds			Seed weight in g		
	$\bar{x} \pm s_{\bar{x}}$	in %	P	$\bar{x} \pm s_{\bar{x}}$	in %	P	$\bar{x} \pm s_{\bar{x}}$	in %	P
				Yield characteristic per single plant					
6.25	59.7 ± 3.7	293.04	++	100.5 ± 7.2	297.39	++	22.12 ± 1.45	324.36	+++
11.11	44.1 ± 1.6	216.19	+++	76.6 ± 1.6	226.71	+++	15.28 ± 0.73	224.08	++
25[a]	20.4	100.00		33.8	100.00		6.82	100.00	
100	4.6 ± 0.3	22.59	+++	7.5 ± 1.3	22.11	+++	1.25 ± 0.17	18.35	++
				Yield characteristic per m^2					
6.25	357.1 ± 7.4	47.10	+++	581.6 ± 42.3	46.47	+++	129.84 ± 7.07	51.38	+++
11.11	490.9 ± 15.9	64.73	+++	857.5 ± 39.5	68.51	++	170.83 ± 4.54	67.58	+++
25[a]	758.3	100.00		1251.6	100.00		252.73	100.00	
100	492.0 ± 13.9	64.88	+++	771.8 ± 17.0	61.67	+++	133.46 ± 9.32	52.81	++

[a]Standard

important for the selection of soybeans for dense planting.

The F_2 is grown at 40 X 11 cm; this is about 23 plants per m^2 and is comparable with the spacing of 20 X 20 cm. To reach the yield target of 3000 kg/ha (45 bu/ac), the F_1 has to yield 27 g per single plant and the F_2 has to yield 13.2 g per single plant.

Deduction of the spacing problem: The trials will be continued; from investigations with different other crops it is concluded that the tendency to more plants per area will continue. It is necessary to grow soybeans with different spacings for screening new varieties or own breedings. Breeders have to consider the requirements of closer spacings.

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1) A spontaneous mutant at the st_2 locus.

In 1971, Detroy Green, Department of Agronomy, Iowa State University, found sterile plants in an F_4 single-plant progeny row from a cross of Hark X Harosoy Dt_2Dt_2. This family segregated 66 fertile to 21 sterile plants. Microspore mother cells of the sterile plants were examined, and a low level of chromosome pairing was observed, indicating that the sterile was either an asynaptic or desynaptic mutant. We designated this new mutant the ISU sterile.

Among F_2 families, we found 18 segregating and 9 nonsegregating families which fit a 2:1 ratio. Furthermore, a ratio of 3 fertile : 1 sterile plants was observed in the 18 segregating families (Table 1, 1972 data).

Segregation for the ISU sterile was observed in other genetic backgrounds as well (Table 1, 1976 data). Crosses were made using plants heterozygous for the ISU sterile and homozygous fertile plants of T241, T242, and T258, respectively. We observed a total of 3284 fertile : 1119 sterile plants (a 3:1 ratio). On the basis of these data, we concluded that sterility in the ISU sterile is controlled by a single gene in the homozygous recessive condition.

Three nonallelic asynaptic or desynaptic mutants have been previously reported in soybeans. Hadley and Starnes (1964) reported $\underline{st}_2$ (T241) and $\underline{st}_3$ (T242). Palmer (1974) later described $\underline{st}_4$ (T258).

The purpose of this study was to determine if this new asynaptic or desynaptic mutant, the ISU sterile, $\underline{st}_?$, is allelic to either $\underline{st}_2$, $\underline{st}_3$, or $\underline{st}_4$. This was done by crossing known heterozygotes, i.e., $\underline{St}_2\underline{st}_2 \times \underline{St}_?\underline{st}_?$, $\underline{St}_3\underline{st}_3 \times \underline{St}_?\underline{st}_?$, and $\underline{St}_4\underline{st}_4 \times \underline{St}_?\underline{st}_?$. F_1 and F_2 populations of each cross were observed. If two lines were allelic with regard to their sterility, then one out of four F_1 plants would be sterile; in the F_2 generation nonsegregating families and families segregating 3 fertile : 1 sterile plants would be observed. If different genes were controlling sterility in the two lines, however, no sterile plants would be observed in the F_1 generation. Moreover, the F_2 generation would include nonsegregating families, families segregating 3 fertile : 1 sterile plants, and families segregating 9 fertile : 7 sterile plants.

In conducting an allelism test with T241H and the ISU sterile, 94 F_1 plants were obtained from 30 different parental combinations. Among these, 66 fertile and 28 sterile plants were observed which fit a 3:1 ratio. Of the 62 F_2 families, 15 were nonsegregating and 47 segregated 3384 fertile : 1197 sterile plants (a 3:1 ratio). No F_2 families segregated 9:7 (Table 2). The F_1 and F_2 data show that T241 and the ISU sterile are genetically alike with regard to their sterility.

Since $\underline{st}_2$, $\underline{st}_3$, and $\underline{st}_4$ previously have been reported to be nonallelic, and $\underline{st}_?$ was found to be allelic to $\underline{st}_2$, it must then be nonallelic to $\underline{st}_3$ and $\underline{st}_4$. To be certain of this we simultaneously conducted allelism tests for $\underline{st}_3$ and $\underline{st}_4$ with $\underline{st}_?$. When crosses were made between T242H and the ISU

Table 1

Ratios of fertile to sterile plants in segregating families of the ISU sterile (1972) and in segregating families of the ISU sterile crosses (1976)

Year	Fertile plants	Sterile plants	χ^2(3:1)	P
1972	434	167	2.49	<.250
1976†	1676	564	0.04	<.900
1976††	633	200	0.44	<.750
1976†††	541	188	0.24	<.750
Totals	3284	1119	3.21	<.750
Pooled χ^2 (1 df)			0.40	<.750
Homogeneity χ^2 (3 df)			2.81	<.500

†, ††, †††Segregating F_2 families from crosses between heterozygous plants of the ISU sterile and homozygous fertile plants of T241, T242, and T258, respectively.

Table 2

Ratios of fertile to sterile plants in the F_1 population and 47 segregating F_2 families from crosses between heterozygous plants of T241 and heterozygous plants of the ISU sterile

Generation	Fertile plants	Sterile plants	df	χ^2(3:1)	P
F_1 Totals	66	28	1	1.15	<.500
F_2 Totals	3384	1197	47	33.72	<.950
Pooled χ^2			1	3.12	<.100
Homogeneity χ^2			46	30.60	<.975

sterile, 38 F_1 plants were obtained from 14 different parental combinations. All 38 F_1 plants were fertile. In the F_2 population, 15 families were all fertile, 13 families segregated 868:310 (3:1), and the remaining 10 families segregated 666:505 (9:7) (Table 3). These observations support the hypothesis

Table 3

Ratio of fertile to sterile plants in segregating[a] F_2 families from crosses between heterozygous plants of T242 and heterozygous plants of the ISU sterile

	Fertile plants	Sterile plants	df	χ^2(3:1)	P	Fertile plants	Sterile plants	df	χ^2(9:7)	P
Totals	868	310	13	13.40	<.500	666	505	10	6.94	<.750
Pooled χ^2			1	1.09	<.500			1	0.19	<.750
Homogeneity χ^2			12	12.31	<.500			9	6.76	<.750

[a]13 families appeared to segregate 3:1 and 10 families 9:7.

Table 4

Ratio of fertile to sterile plants in segregating[a] F_2 families from crosses between heterozygous plants of T258 and heterozygous plants of the ISU sterile

	Fertile plants	Sterile plants	df	χ^2(3:1)	P	Fertile plants	Sterile plants	df	χ^2(9:7)	P
Totals	1166	411	17	14.52	<.750	721	575	12	8.06	<.900
Pooled χ^2			1	0.95	<.500			1	0.20	<.750
Homogeneity χ^2			16	13.57	<.750			11	7.86	<.750

[a]17 families appeared to segregate 3:1 and 12 families 9:7.

that sterility in T242 and the ISU sterile are controlled by two different genes.

In the allelism test between T258H and the ISU sterile, we obtained 38 F_1 plants from 15 different parental combinations. We found no sterile plants among the F_1 plants. The F_2 population included 9 families that did not segregate, 17 families that segregated 1166:411 (3:1), and 12 families that segregated 721:575 (9:7) (Table 4). We, therefore, concluded that sterility in T258 and the ISU sterile are controlled by two different genes.

Three nonallelic asynaptic or desynaptic mutants have previously been reported in soybeans, $\underline{st}_2$(T241), $\underline{st}_3$(T242), and $\underline{st}_4$(T258). The sterile found by Detroy Green was also an asynaptic or desynaptic mutant. It was shown to be allelic with $\underline{st}_2$ on the basis of sterile plants in the F_1 and no 9:7 ratios in the F_2 populations. On the other hand, it was found to be nonallelic to $\underline{st}_3$ and $\underline{st}_4$ because there were no sterile F_1 plants, and there were 9:7 ratios in the F_2 population.

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2) Soybean linkage tests.

In 1969, Walter Fehr of Iowa State University found a dwarf soybean plant (T263) in an early elite breeding population. Allelism tests have not been conducted with the other dwarfs, $\underline{df}_2$, $\underline{df}_3$, and $\underline{df}_4$. We have used this early elite dwarf mutant in crosses with our trisomics.

Segregation ratios were determined for the F_2 progenies from disomic and trisomic plants (Table 1). Previous tests indicated that genes controlling pubescence color ($\underline{T}_1\underline{t}_1$) and flower color ($\underline{W}_1\underline{w}_1$) were not on trisomics A, B, or C. In crosses with trisomics A and B, we classified F_2 progenies for plant height, flower color and pubescence color (Table 2). F_2 linkage results are presented with a = XY, b = Xy, c = xY and d = xy for the gene pairs listed in the form of Xx and Yy. Percentage recombination was obtained from the ratio of

Table 1

Segregation ratios of F_2 disomic and trisomic progenies for tall and dwarf plants

Chromosome type	Number of plants		Ratio
	Tall	Dwarf	
Disomic A	262	92	2.85:1
Trisomic A	358	97	3.69:1
Disomic B	323	120	2.69:1
Trisomic B	345	103	3.35:1
Disomic C	112	34	3.29:1
Trisomic C	327	116	2.82:1

Table 2

F_2 linkage tests

Genes	General phenotypic classes				Sum	%R ± SE	Linkage phase
	a	b	c	d			
	T263 ($\underline{W}_1\underline{t}_1$ dwarf) X ($\underline{w}_1\underline{T}_1$ tall)†						
$\underline{W}_1\underline{w}_1$ $\underline{T}_1\underline{t}_1$	950	301	320	129	1700	53.4 ± 1.8	repulsion
$\underline{W}_1\underline{w}_1$ tall dwarf	952	299	336	113	1700	51.0 ± 1.8	repulsion
$\underline{T}_1\underline{t}_1$ tall dwarf	1158	112	130	300	1700	15.4 ± 1.0	coupling
	T266 ($\underline{ms}_1\underline{W}_1$) X ($\underline{Ms}_1\underline{w}_1$)†						
$\underline{W}_1\underline{w}_1$ $\underline{Ms}_1\underline{ms}_1$	1268	557	583	60	2468	30.4 ± 1.8	repulsion
	T260 ($\underline{ms}_1\underline{w}_1$) X ($\underline{Ms}_1\underline{W}_1$)††						
$\underline{W}_1\underline{w}_1$ $\underline{Ms}_1\underline{ms}_1$	451	91	87	100	729	27.9 ± 2.0	coupling

†Includes disomics and trisomics A and B.

††Includes disomic and trisomic C.

products following the method of Immer and Henderson (1943).

The dwarf trait is not located on trisomics A, B or C, nor is it linked to $\underline{W}_1\underline{w}_1$. It is linked to $\underline{T}_1\underline{t}_1$ of Linkage Group 1, with 15.4% recombination.

Additional tests are underway with the other mutants of Linkage Group 1.

In another linkage study, involving flower color ($\underline{W_1}\underline{w_1}$) and male sterility ($\underline{Ms_1}\underline{ms_1}$), we noticed linkage of $\underline{Ms_1}\underline{ms_1}$ and $\underline{W_1}\underline{w_1}$ and the results show 27.9% and 30.4% recombination for coupling and repulsion phase, respectively (Table 2).

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1) Observation on cross-pollination of soybeans after gamma irradiation of seeds.

Seeds of 'Sansai' variety, line No. 34-9-1 (white flower, as a female parent) were irradiated with gamma rays of a cesium^{137} source; 5 and 15 krad were used. After treatment, Sansai seeds were grown in alternate rows with S.J. 1 line No. 56-12 and S.J. 2 line No. 27-9 (both purple flowers, as male parents). The experiments were carried out in replications on Kasetsart campus and at Suwan Farm, Pakchong. M_1 plants of Sansai were singly harvested and threshed separately. M_2 seeds of Sansai were then sown in rows (plant-to-row). Observations on flower color of M_2 plants were carefully made. Sansai plants with purple flowers were found in both experiments as shown in Table 1.

In a combination of Sansai and S.J.1, on Kasetsart campus, 1 plant was found among 2741 M_2 plants derived from 15 krad treatment. None was found among M_2 plants derived from either control or 5 krad treatment. In a combination of Sansai and S.J.2, on Kasetsart campus, 2 plants were found among 2412 M_2 plants derived from 15 krad treatment. None was found among M_2 plants derived from either control or 5 krad treatment.

In a combination of Sansai and S.J.1 at Suwan Farm, Pakchong, 1 plant was found among 2385 M_2 plants derived from 15 krad treatment. None was found among M_2 plants derived from either control or 5 krad treatment. In a combination of Sansai and S.J.2 at Suwan Farm, 2 and 6 plants were found among 3226 and 3252 M_2 plants derived from 5 and 15 krad treatments, respectively.

Table 1

Field hybrids of the soybean after gamma irradiation of seeds

Dose krad	M_1 Sansai (♀) plants		M_2 plants		Purple-flowered plants obtained	
	S.J.1 (♂)	S.J.2 (♂)	S.J.1	S.J.2	S.J.1	S.J.2
	a. Location: Kasetsart campus, Bangkok					
0	30	30	3095	2745	0	0
5	30	30	2850	2483	0	0
15	30	30	2741	2412	1	2
	b. Location: Suwan Farm, Pakchong					
0	32	32	2940	3769	0	0
5	32	32	2134	3226	0	2
15	32	32	2385	3252	1	6

None was found among M_2 plants derived from control.

Seeds of 12 field hybrid plants of both combinations were grown in rows (plant-to-row) on Kasetsart campus in order to prove of true hybrid plants. Segregation of flower colors (purple : white) in a ratio of approximately 3:1 was obtained from the 12 plants. It was concluded that all 12 plants were true hybrids.

Pollen and pollen tube growth of Sansai after seed treatment with gamma rays were studied in comparison with pollen and pollen tube growth of Sansai, S.J.1 and S.J.2 without seed irradiation. It was found that, after 1½ hr in artificial media, 41-45% of pollen of treated Sansai were germinated, while 70-81% germination was obtained in nontreated varieties.

In conclusion, we were not able to detect the natural outcrossing frequency in Sansai soybean variety in this experiment; this might be: (1) Sansai is a very highly self-pollinated variety, and (2) the number of initiated plants (30-32 plants) taken for evaluation was too small. Anyhow, with the same number of M_1 plants, we were able to detect 0.04-0.18% of outcrossing in the 15 krad treatment. Though the data are much less than 14%, the highest frequency reported by Beard and Knowles (1971) in their soybean irradiation experiment with honeybees, the data support the recommendation of growing

irradiated materials in isolation from breeding lines or other varieties, in order to avoid outcrossing as well as a question of whether it is a true mutant or a field hybrid.

References

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1) Recurrent selection for yield in soybeans.

Recurrent selection procedures have had limited application in soybean improvement (Kenworthy, 1976). Despite the procedural difficulties, theoretical considerations suggest an advantage for recurrent selection in supplementing standard breeding procedures. We present here a preliminary report of the yield response realized from several cycles of selection. In this study each cycle required two years to complete.

The initial selection population was synthesized by backcrossing nine plant introductions, high in protein and of diverse origin, to a highly productive experimental line, D49-2491. Fifty-five BC_1S_1 progenies (designated C_0) were chosen from a test population of 495 as parents for the first cycle of selection. At least one S_1 progeny from each of the nine backcrosses was included in C_0. The 55 progenies were randomly intermated to generate a test population of 431 C_1S_1 progenies from which 20 lines were chosen for the second cycle (C_2) of intermating. Thereafter, 20 S_1 progenies, chosen from test populations of about 200, were used as parents for C_2 and C_3. In each cycle, parents for intermating were chosen on the basis of yield performance in

replicated nine-hill plots (Schutz and Brim, 1967) grown at only one location. Remnant seed from each of the selected S_1 progenies were advanced to the S_3 generation for testing. Equal quantities of seed from each selected progeny within cycles were pooled to form an entry in the tests to determine selection response. Thus, the yield trial consisted of 5 entries: C_0, C_1, C_2, C_3 and D49-2491. Only 20 S_1 progenies of the 55 selected for C_0 were advanced for evaluation in this comparison. These 20 progenies represented the highest yielding lines of the 55 selected for the first intermating. The entries were tested in 3-row plots, 3.0 m long. Spacing between rows of the plot was 48 cm and the entire plot was harvested. The experiment was grown at one location in 1973 and at three locations in 1974 in a randomized block design with 12 replications at each location.

The yield responses obtained from recurrent selection expressed as a percent of C_0 are shown below:

Entry	Yield Response
D49-2491	97
C_0	100
C_1	110
C_2	112
C_3	116

A significant linear increase in yield was obtained. The average increase in yield from C_0 to C_3 was 134 kg/ha/cycle; the cumulative gain was 427 kg/ha. C_3 yielded 16% and 19% higher than C_0 and D49-2491, respectively. The greatest yield response was obtained in C_1.

The yield responses obtained are conservative estimates for two reasons. First, C_0 was represented by only 20 of the highest yielding progenies of the 55 chosen for generating C_1. A less conservative test would have included all 55 of the progenies. Second, the recurrent parent or a composite of the 495 backcross progenies from which C_0 was selected could be used as controls for these comparisons. However, the use of C_0 as the control provides a more conservative test than the other possible alternatives.

It is encouraging that yield performance in nine-hill plots was effective in identifying superior parents for intermating. This is especially true when one considers that these evaluations were conducted at only one location. Furthermore, this location was not used as a test site in determining the

selection response reported here. The favorable yield response observed in this population suggests that recurrent selection is a viable scheme for the development of populations of greater diversity as well as productivity.

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1) Field screening of soybean germplasm (Maturity Groups 00 to IV) against *H. zea* damage.*

Genetic base of major commercial soybean cultivars is very narrow. The top 10 most widely grown cultivars in the U.S. have been developed from 17 parent lines (Hartwig, 1973). Recently three soybean Plant Introductions, namely, PI 171.451, PI 227.687 and PI 229.358, have been found to have good leaf-feeding resistance to Mexican bean beetle (*Epilachna varivestis* Mulsant) (Van Duyn *et al.*, 1972) and corn earworm (*Heliothis zea* Boddie) (Hatchett *et al.*, 1976; Joshi and Wutoh, 1976). Most of the plant breeders are using these three PI's very extensively in developing varieties resistant to various insect pests of soybeans. This practice may again result in a narrow genetic base of resistant cultivars. The objective of this investigation was to screen soybean germplasm from Maturity Groups 00 to IV for resistance to *H. zea* under field conditions.

Materials and methods: During 1974, 30 seeds of each germplasm entry and some advanced breeding lines, totaling 2797, were planted in the field in rows 36" long and 36" apart. Resistant germplasm with yellow seed coat (550 entries) was again planted during 1975. Field plantings were made up to May 28 during 1974 and on May 28 during 1975. Pods of each cultivar were examined for *H. zea* damage at maturity. Cultivars with no damaged pods were classified

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as resistant.

Results and discussions: Out of 2797 germplasm entries tested in the field, 625 (22%) were not damaged by this pest during 1974. Out of 625 germplasm entries, 550 cultivars were again tested in the field during 1975. It was found that only 461 out of 550 were not damaged by this pest for two years. The list of resistant germplasm follows:

Maturity Group 00

Ada	PI 377.422	PI 189.877	PI 194.639
Portage	PI 372.406	PI 189.880	PI 194.643
PI 360.962	PI 153.293	PI 189.886	PI 194.644
PI 347.540[A]	PI 154.198	PI 153.314	PI 194.645
PI 347.540[B]	PI 180.507	PI 189.906	PI 194.647
PI 361.068	PI 180.508	PI 189.932	PI 196.485
PI 361.078	PI 180.509	PI 189.937	PI 196.486
PI 361.086	PI 180.516	PI 194.624	PI 196.491
PI 361.107	PI 180.517	PI 194.627	PI 196.504
PI 361.108	PI 180.519	PI 194.630	PI 198.067
PI 372.403[B]	PI 180.525	PI 194.632	PI 240.079

Forty-four cultivars/PI's were found to be resistant in Maturity Group 00.

Maturity Group 0

Capital	PI 347.568	PI 153.261	PI 370.402
Grant	PI 347.570	PI 161.989	PI 372.403[C]
PI 290.133	PI 153.213	PI 290.116[A]	PI 372.424
PI 297.507[B]	PI 361.061[A]	PI 347.567	FC 30.684
PI 297.513	PI 361.077[B]	PI 154.189	PI 189.898
PI 347.559[B]	PI 361.091	PI 189.897	PI 189.900
FC 30.684	PI 181.531	PI 347.559[A]	PI 189.913
PI 68.722	PI 181.571	PI 361.120	PI 204.652
PI 70.242-4	PI 189.882	PI 370.057[A]	PI 227.330
PI 153.259	PI 189.893	PI 370.058	PI 238.924

Forty cultivars/PI's were found to be resistant in Maturity Group 0.

Maturity Group I

Blackhawk	PI 358.319	PI 68.770	PI 88.295
Bombay	PI 362.092	PI 70.016	PI 88.443
Disoy	PI 361.095	PI 70.017	PI 88.805-2
Earlyana	PI 361.098	PI 70.087	PI 89.055
Habaro	PI 361.104	PI 70.473-1	PI 153.263
PI 291.294	PI 370.059	PI 70.520	PI 153.283
PI 291.303[A]	PI 54.853	PI 71.161	PI 181.536
PI 297.514	PI 68.551-3	PI 70.241	PI 181.538
PI 342.437	PI 68.554	PI 79.699	PI 153.308
PI 347.546[B]	PI 68.572	PI 81.037-4	PI 189.966
PI 358.315[A]	PI 68.586	PI 81.775	PI 227.322

Maturity Group I (cont'd)

PI 361.066[A]	PI 68.610	PI 84.686	PI 227.329
PI 361.066[B]	PI 68.746	PI 84.964	PI 189.916
PI 361.087		PI 86.416	

Fifty-four cultivars/PI's were found to be resistant in Maturity Group I.

Maturity Group II

Amsoy	PI 68.670-1	PI 68.788	PI 73.583
Beeson	PI 68.670-2	PI 68.795	PI 73.585
Corsoy	PI 68.671	PI 69.500	PI 79.613
Goku	PI 68.686	PI 69.512	PI 79.862-1
Harosoy 63	PI 68.680	PI 70.009	PI 79.863
Harwood	PI 68.687	PI 70.021	PI 84.965
Linman 533	PI 68.694	PI 70.036	PI 85.508
Madison	PI 68.704	PI 70.077	PI 86.741
Provar	PI 68.706	PI 70.078	PI 86.878
SRF 200	PI 68.708	PI 70.084	PI 88.293[A]
PI 248.396	PI 68.709	PI 70.197	PI 88.294-1
PI 253.650[B]	PI 227.334	PI 70.224	PI 88.301
PI 68.516	PI 266.085[C]	PI 70.228	PI 88.355
PI 68.521	PI 291.290	PI 70.242	PI 88.803
PI 68.522	PI 291.291	PI 70.457	PI 88.810
PI 68.526	PI 291.296	PI 70.459	PI 71.850
PI 68.530	PI 291.298	PI 70.461	PI 88.495
PI 68.543	PI 291.309[A]	PI 70.463	PI 89.004
PI 61.551-2	PI 347.539[A]	PI 70.503	PI 89.072
PI 68.555	PI 347.539[B]	PI 70.516	PI 91.132-2
PI 68.564	PI 360.840	PI 361.065[B]	PI 91.167
PI 68.587	PI 68.712	PI 361.080	PI 92.465
PI 68.598	PI 68.713	PI 361.074	PI 92.583
PI 68.600	PI 68.715	PI 361.109	PI 92.611
PI 68.609[LB]	PI 68.718	PI 361.116	PI 92.677
PI 68.622	PI 68.725	PI 370.057[B]	PI 92.683
PI 68.627	PI 68.728	PI 54.604	PI 92.719
PI 68.629	PI 68.729	PI 68.475	PI 181.537
PI 68.639	PI 68.762	PI 68.475-1	PI 189.930
PI 68.642	PI 68.765	PI 68.500	PI 200.479
PI 68.658	PI 68.778	PI 68.503	PI 227.321
PI 68.661		PI 68.508	

One-hundred and twenty-six cultivars/PI's were found to be resistant in Maturity Group II.

Maturity Group III

Adelphia	SRF 350	PI 68.535	PI 70.541
AK (Harrow)	PI 253.660[A]	PI 68.621	PI 70.566
Bavender Special A	PI 283.331	PI 68.679	PI 70.461
Bavender Special B	PI 291.286	PI 68.701	PI 71.845
Chusei	PI 291.310[C]	PI 68.710	PI 88.291
Dunfield	PI 297.504	PI 68.731	PI 88.297

Maturity Group III (cont'd)

Harman	PI 361.063	PI 68.732-1	PI 88.303-1
Illington	FC 02.108	PI 68.748-1	PI 88.306
Jogun (Ames)	PI 68.398	PI 68.761-3	PI 88.312
Lincoln	PI 68.423	PI 68.806	PI 88.349
Manchu Lafayette	PI 68.494	PI 90.463	PI 88.353
Ross	PI 68.521-1	PI 90.566	PI 88.354
Viking	PI 79.797	PI 89.150	PI 89.002
PI 69.515	PI 79.835	PI 90.578	PI 89.005-4
PI 69.995	PI 79.872	PI 91.153	PI 89.061-2
PI 70.001	PI 79.848-1	PI 92.602	PI 89.066
PI 70.014	PI 80.822	PI 92.608	PI 90.392
PI 70.023	PI 82.232	PI 92.617	PI 181.554
PI 70.076	PI 84.682	PI 92.623	PI 153.309
PI 70.080	PI 84.908-2	PI 98.243	PI 189.920
PI 70.188	PI 85.668	PI 157.457	PI 196.148
PI 70.192	PI 85.878	PI 157.491	PI 196.156
PI 70.199	PI 86.026-1	PI 70.462	PI 196.157
PI 70.201	PI 86.123	PI 70.470	PI 200.453
PI 70.202	PI 87.574	PI 70.471	PI 200.457
PI 70.212	PI 87.634	PI 70.473	PI 200.480
PI 70.213	PI 238.334	PI 70.500	PI 200.548
PI 70.247	PI 68.530-2	PI 70.501	PI 226.588
PI 70.019	PI 68.533-1	PI 70.519	PI 227.560
PI 89.012-1	PI 68.533-2	PI 70.528	PI 227.686
PI 243.532	PI 88.282	PI 92.618	PI 235.339
Williams			

One-hundred twenty-five cultivars/PI's were found to be resistant in Maturity Group III.

Maturity Group IV

Bethel	PI 243.525	PI 54.617	PI 92.689
Bonus	PI 243.528	PI 61.944	PI 157.459
Clark	PI 340.017	PI 62.248	PI 172.901
Clark 63	PI 246.366	PI 68.644	PI 181.550
Cutler 71	PI 246.367	PI 68.679-2	PI 181.557
Cypress No. 1	PI 253.651[A]	PI 70.208	PI 157.419
Fabulin	PI 253.651[B]	PI 71.444	PI 157.437
Hokkaido	PI 253.651[D]	PI 71.506	PI 157.452
Harbinsoy	PI 71.463	PI 79.825-1	PI 200.470
Higan	PI 253.652[B]	PI 84.960	PI 200.536
Kailua	PI 253.654	PI 86.876	PI 205.088
Kaikoo	PI 253.656[A]	PI 87.631-3	PI 226.591
Macoupin	PI 253.661[C]	PI 88.302-1	PI 228.064
Makapu Summer	PI 266.806[D]	PI 88.499	PI 229.314
Perry	PI 274.210	PI 88.814	PI 229.319
Roe	PI 340.010	PI 88.820[N]	PI 229.325
Scioto	PI 340.012	PI 89.010	PI 235.335
Scott	PI 360.845	PI 89.128	PI 235.346
SRF 450	PI 361.103	PI 90.492-2	PI 235.651[B]

Maturity Group IV (cont'd)

SRF 425	FC 03.654-1	PI 91.133	PI 90.402
PI 243.514	FC 19.979-3	PI 91.731-1	

Eighty-three cultivars/PI's were found to be resistant in Maturity Group IV.

Recent data from other field experiments at UMES (unpublished) and by other investigators (Deitz et al., 1976) indicate that early planted soybeans escape damage from this pest. Therefore, some of these cultivars might have shown resistance through escape mechanism. It is hoped that plant breeders engaged in developing resistant varieties to various insect pests of soybeans may want to look at these germplasm entries more critically.

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J. M. Joshi

2) Mechanism of corn earworm resistance in some soybean cultivars.*

Much emphasis is being placed on the development of new soybean (Glycine max (L.) Merrill) cultivars resistant to various insect pests. Three soybean PI's (171.451, 229.358, 227.687) have been found to have antibiosis to Mexican

*This is part of a CSRS/USDA funded project.

bean beetle (Epilachna varivestis Mulsant) (Van Duyn et al., 1972) and corn earworm (Heliothis zea Boddie) (Clark et al., 1972; Hatchett et al., 1976; Joshi and Wutoh, 1976). These PI's also have exhibited resistance to bean leaf beetle (Cerotoma trifurcata Foster); cultivars 'Shore' and 'Wye' also were observed to have leaf feeding resistance to this pest (Joshi and Wutoh, 1976). The objective of this study was to investigate mechanism of resistance in Shore, Wye and 'ED 73-371' to H. zea.

Materials and methods: Six cultivars ('Davis', PI 229.358, PI 227.687, Wye, Shore, ED 73-371) were planted in the field on May 17, 1976. Davis (Beland and Hatchett, 1976; Hatchett et al., 1976) and an artificial diet (supplied by Bio-Serv, Frenchtown, NJ) were susceptible checks, and two PI's (229.358, 227.687) were resistant checks. On July 26, 1976, when plants were in 7th to 9th trifoliolate stage, foliage feeding was started. Three newly-hatched larvae (<24 hours old) were placed in each cup (50 cups/treatment) along with a leaflet from the first fully expanded trifoliolate of each cultivar. Sufficient artificial diet was placed in each cup in the beginning and more was added when necessary for artificial diet treatment. After 72 hours, larvae were thinned out to one/cup. Mean minimum and maximum temperatures during experimentation were 74.4°F and 76.6°F. Larval weight (after 10 and 15 days), pupal weight (on 6th day after pupation), length of larval and pupal stage were recorded. Larvae were checked for mortality daily. Other techniques of feeding and rearing were the same as reported in an earlier publication (Joshi and Wutoh, 1976).

Results and discussion: The effect of different feeding treatments on some growth parameters is given in Table 1. H. zea larvae reared on artificial diet had maximum larval and pupal weight, shortest larval and longest pupal stage, and lowest mortality as compared with other treatments, whereas PI 227.687 offered maximum leaf feeding resistance with lowest larval and pupal weights, greatly extended larval stage and maximum mortality. Larval and pupal mortality on PI 227.687 was 36%. This mortality is very low as compared with Hatchett et al. (1976) who have reported 100% mortality on this PI. It appears that environments under which cultivars are grown and feeding test conducted, have profound effect on the expression of antibiosis.

Shore and PI 227.687 exhibited more antibiosis after 10 days feeding as expressed in low larval weight than any other treatment. Larvae reared on Wye, PI 229.358 and ED 73-371 also gained less weight than those reared on

Table 1

Development of *Heliothis zea* larvae on synthetic diet and leaves of different soybean cultivars or PI's

Treatment[†]	$\bar{x}$ (mg) Larval wt. after 10 days	15 days	$\bar{x}$ (mg) Pupal wt.[††]	Days in larval stage	Days in pupal stage	Larval mortality 1-10th day	11-15th day	16th-pupation	Total larval mort.	pupal mort.	Total mort. (%)
Control (synthetic diet)	631a*	----	432a*	14.9e*	14.5a*	2	0	0	2	2	8
Davis	225b	454b*	315c	17.8d	13.1b	3	1	2	6	2	16
ED 73-371	145c	534a	345b	18.9c	13.3b	4	2	1	7	4	22
PI 229.358	135c	529a	360b	18.9c	13.1b	5	0	2	7	4	22
Wye	134c	503ab	342b	19.6bc	13.0b	3	0	4	7	4	22
Shore	89d	445b	315c	19.9b	12.8b	4	0	4	8	2	20
PI 227.687	70d	213c	274d	23.7a	13.1b	13	2	1	16	2	36

*Means not followed by the same letter were significantly different at the 0.05 probability level according to Duncan's Multiple Range Test.

[†]50 larvae/treatment.

[††]Mean weight of pupae on sixth day after pupation.

Davis in the same feeding period. However, larval weight after 15 days indicated no difference among ED 73-371, PI 229.358 and Wye; although the larvae reared on these cultivars gained more weight than on Davis, Wye and Shore. It appears that with the exception of Davis, larvae ≤10 days old were unable to feed satisfactorily on these cultivars, whereas no leaf feeding resistance was observed for any other cultivar except PI 227.687 when the larvae were 11 to 15 days old.

Leaf feeding resistance to H. zea, up to first 10 days, in ED 73-371, PI 229.358, Wye and Shore may be biophysical in nature but the antibiosis expressed by PI 227.687 may be due to nutritional deficiency or nutrient disproportionality as indicated by low larval weight and extended larval stage.

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J. M. Joshi

3) Screening soybean germplasm for multiple pest resistance.*

Use of resistant soybean cultivars is an excellent method of controlling various insect pests and diseases. A resistant plant has built-in protection which lasts throughout the growing season. Cultivars having resistance to

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only one pest may still need chemical control measures for protection from other pests. However, development of cultivars with multiple pest resistance can eliminate the use of poisonous chemicals. Downy mildew caused by Peronospora manshurica (Naoum) is one of the serious soybean diseases which can reduce yield by 8% (Athow, 1973) and among the insect pests, corn earworm (Heliothis zea Boddie) is one of the most destructive pests of this crop. Corn earworm infestation sometimes can cause complete crop loss (Turnipseed, 1973).

The present investigation was undertaken to screen maturity group V germplasm for downy mildew and corn earworm resistance under field conditions.

Materials and methods: Fifty seeds of each of the 248 cultivars/PI's were planted in the field June 5, 1975. Cultivars/PI's were examined for pod damage by corn earworm. Soybean cultivars which had one or more damaged pods were classed as susceptible and the others without any damage were classified as resistant. All cultivars were also checked for downy mildew infestation Sept. 3, 1975.

Experimental results and discussion: Soybean cultivars which did not show any symptoms of downy mildew are as follows:

Dorman	PI 123.577	PI 238.929
Dortchsoy 67	PI 157.413	PI 238.932
Arlington	PI 157.443	PI 274.422
Hollybrook	PI 157.444	PI 274.508
Luthv	PI 157.451	Dixie
Austin	PI 157.470	Lexington
FC 30.265	PI 157.473	PI 303.652
FC 31.719	PI 157.493	PI 319.527
FC 31.934	PI 170.896	PI 319.528
FC 31.952	PI 171.430	PI 319.532
PI 60.269	PI 171.442	PI 339.867
PI 60.273	PI 179.823	PI 339.869
PI 60.296	PI 179.825	PI 381.670
PI 62.203	PI 181.543	PI 339.980
PI 62.204	PI 181.547	PI 339.989
PI 71.465	PI 181.562	PI 339.992
PI 71.677	PI 187.155	PI 339.998
PI 79.832	PI 196.166	PI 339.999
PI 80.466	PI 200.447	PI 340.000
PI 81.042	PI 200.450	PI 340.003
PI 81.780-S	PI 200.468	PI 340.004
PI 82.286	PI 200.495	PI 340.006
PI 82.588	PI 200.503	PI 340.008
PI 83.836	PI 200.510	PI 340.013
PI 83.942	PI 200.534	PI 340.014
PI 84.632-S	PI 200.546	PI 340.016

PI 84.734	PI 209.333	PI 340.026
PI 84.910	PI 210.179	PI 340.029
PI 85.089	PI 219.780	PI 340.043
PI 85.252	PI 219.785	PI 340.045
PI 86.045-S	PI 219.789	PI 342.003
PI 86.465	PI 221.973	PI 346.306
PI 86.982	PI 227.158	PI 346.309
PI 87.037	PI 227.159	PI 355.067
PI 88.490	PI 227.555	PI 355.069
PI 88.820	PI 227.567	PI 355.070
PI 89.061	PI 229.315	PI 371.610
PI 89.154-S	PI 229.335	PI 371.611
PI 90.243	PI 229.337	PI 381.659
PI 90.481	PI 229.346	PI 381.663
PI 91.725	PI 229.350	PI 381.655
PI 95.959	PI 235.347	PI 381.668
PI 96.169	PI 238.928	

One-hundred twenty-eight out of 248 were found to be resistant to downy mildew during 1975 screening program.

Corn earworm resistance: Cultivars which did not sustain any pod damage from corn earworm are listed as follows:

Dorman	PI 157.394	PI 340.009
Dortchsoy 67	PI 157.451	PI 340.019
Harrel	PI 157.470	PI 340.044
Arlington	PI 170.893	PI 340.051
Nansemond	PI 181.544	PI 342.002
Peking	PI 181.546	PI 342.003
S-100	PI 181.558	PI 371.610
FC 30.265	PI 196.177	PI 371.611
FC 31.683	PI 200.450	PI 381.662
FC 31.721	PI 235.347	PI 381.664
PI 60.273	PI 238.928	PI 381.666
PI 62.203	PI 274.422	PI 381.667
PI 65.342	Lexington	PI 381.670
PI 71.465	PI 322.693	PI 381.671
PI 79.832	PI 322.694	PI 381.673
PI 81.780-S	PI 324.924	PI 381.674
PI 82.588	PI 346.307	PI 381.675
PI 83.942	PI 346.308	PI 381.676
PI 87.542	PI 339.866	PI 381.677
PI 95.959	PI 339.978	PI 381.678
PI 96.089	PI 339.982	PI 381.684
PI 96.786	PI 339.998	

Sixty-five cultivars/PI's out of 248 were observed to be resistant to corn earworm under field conditions during 1975.

Multiple resistance: Cultivars with resistance to both downy mildew and corn earworm are as follows:

Dorman	PI 82.588	Lexington
PI 60.273	PI 157.470	PI 371.611
PI 81.780-S	PI 274.442	FC 30.265
PI 157.451	PI 371.610	PI 79.832
PI 238.928	Arlington	PI 95.959
PI 342.003	PI 71.465	PI 235.347
Dortchsoy 67	PI 83.942	PI 339.998
PI 62.203	PI 200.450	PI 381.670

Twenty-four cultivars/PI's were found to have field resistance to both of these pests.

The use of diversified germplasm with multiple pest resistance in developing cultivars resistant to various pests will greatly reduce the need of chemical control measures.

Acknowledgements: Sincere thanks are expressed to Dr. J. G. Wutoh, Associate Professor (Biology), UMES and Professor in Charge, Marine Products Laboratory, Crisfield, University of Maryland, who initiated research on this project and secured necessary funding from CSRS/USDA. I am also grateful to Dr. Edgar E. Hartwig, ARS/USDA, Delta Branch Experiment Station, Stoneville, MS, for supplying germplasm and to Mr. Oswald Andrade for his technical assistance.

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1) Evaluation of some soybean isolines in irrigation culture.*

About 9% of the total soybean acreage and about 50% of the total corn acreage in Nebraska was irrigated at least once during the growing season in 1975. The 1975 state averages for irrigated soybeans and irrigated corn were

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2220 and 7605 kg/ha respectively. Obviously, this yield differential (in relation to the price and production cost differentials) accounts for the reason most farmers utilize their irrigated acreage for corn rather than soybeans. It seems reasonable to assume that if irrigated soybean yields of 3500-4000 kg/ha or greater could be consistently obtained, soybeans could become an effective competitor with corn for irrigated land resources. This yield range is certainly not unobtainable as some cultivars in irrigated variety trials often reach the 3500-4000 kg/ha level (Dreier et al., 1975).

Soybean cultivars with a somewhat shorter stature would seem to be the solution to the problem of utilizing irrigation to maintain optimum soil moisture levels throughout the growing season and yet still avoid the lush vegetative growth and the subsequent severe lodging which limits the substantial yield potential often inherent under these conditions (Cooper, 1971). In recent years, there have been several studies on the potential of the dt_1 (determinate) and Dt_2 (semi-determinate) genes with respect to reducing lodging (Cooper, 1976; Chang et al., 1976). Cultivars carrying either one of these genes were shorter in height and consequently, lodging was reduced, but particularly with dt_1. An earlier report (Hicks et al., 1969) also indicated that the gene Dt_2 may confer a slight yield advantage in narrow and conventional row spacings in dryland production even though the lodging scores of the Dt_2 and normal types were not very different.

We are currently interested in evaluating soybean genetic morphological variants in irrigation culture to determine their yield potential and lodging resistance. In 1976, we selected several 'Clark' and 'Harosoy' isolines for a preliminary test under irrigation (original seed provided by R. L. Bernard, USDA-ARS, Urbana, IL). A randomized complete block design with four replications was used. Individual plots consisted of 3 rows, 6.1 m in length, from which a 3.05 m section of the center row was ultimately harvested for yield. The test was irrigated three times during the growing season, July 12, July 29 and August 13. A planned fourth irrigation during the last week in August was not accomplished because of other demands on the water resources during this rather dry year. The isolines used are listed in Table 1. The gene e_2 in Clark was included to provide isolines with a maturity more adapted to the Lincoln area. The gene E_1 in Harosoy was selected because in previous tests it had been extremely sensitive to lodging when grown under irrigation.

The agronomic data shown in Table 1 reveal that the leaf shape genes

Table 1

Agronomic data for some Clark and Harosoy isolines grown under irrigation

Isoline	Line designation	Yield (kg/ha)	Maturity	Lodging	Height (cm)
Clark - +	(L1)	2102 b	10/5	1.5 a	116 a
Clark - $\underline{Dt_2}$	(L62-1251)	2154 b	10/4	1.6 a	84 c
Clark - $\underline{e_2}$	(L62-1932)	2494 a	9/18	1.1 b	100 b
Clark - $\underline{Dt_2e_2}$	(L67-3232)	2845 a	9/18	1.0 b	73 d
Clark - $\underline{ln}$	(L62-1579)	2019 b	10/4	1.5 a	114 a
Harosoy - +	(L2)	2470 a	9/14	1.6 b	104 b
Harosoy - $\underline{Dt_2}$	(L62-361)	2731 a	9/15	1.0 d	77 c
Harosoy - $\underline{E_1}$	(L68-694)	1558 b	10/9	5.0 a	133 a
Harosoy - $\underline{ln}$	(L63-1212)	2473 a	9/14	1.1 cd	105 b
Harosoy - $\underline{lo}$	(L65-372)	2455 a	9/16	1.4 bc	103 b

C.V. = 10.4%

(*ln* and *lo*) were similar to the normal Harosoy isoline in most respects. The Harosoy-E_1 isoline, however, lodged almost completely within a day or two after the second irrigation and its yield was only 60% of the normal isoline. While one cannot ignore the substantial maturity difference between the E_1 and normal Harosoy isolines, the early lodging resulting from the 28% increase in height was probably the primary factor in the yield reduction (compare, for example, the Harosoy-E_1 isoline and the normal Clark isoline which are somewhat more similar in maturity). Although the Harosoy-Dt_2 isoline was shorter (26%) in height, which resulted in significantly less lodging, the 10% yield advantage over the normal isoline was not statistically significant.

Surprisingly, the Clark-Dt_2 isoline did not differ significantly from the normal isoline (or the *ln* isoline) in either yield or lodging, even though it was significantly shorter (28%) in height. This may have been due to the absence of the planned fourth irrigation which for the later-maturing Clark might have masked a potential lodging and yield differential. The Clark-e_2 isoline, which was about two weeks earlier and 14% shorter than the normal isoline, showed significantly greater yield (about 400 kg/ha), probably because of the better maturity 'fit' with the irrigation schedule. Interestingly, the Clark-Dt_2e_2 isoline was 27% shorter and had a nearly significant 14% yield advantage over Clark-e_2, indicating a synergistic effect of Dt_2 and e_2 on yield under the irrigation schedule imposed. Although the lodging scores of the Dt_2 and normal isolines were similar, the corresponding earlier Dt_2e_2 and e_2 isolines were shorter and, consequently, had significantly less lodging.

While we recognize the limitations in the isoline approach to evaluating contrasting alleles (Cooper, 1976), these preliminary data indicate that the reported slight yield advantage of the Dt_2 gene (Hicks *et al.*, 1969) can be enhanced in irrigation culture when present in a genetic background of an appropriate maturity that 'fits' the irrigation schedule imposed. In addition, these data indicate the critical importance of the timing of irrigation with respect to reproductive differentiation and development. In this regard, isolines differing in flowering and maturity dates (i.e., the major maturity genes) may be of significant use in identifying those critical, perhaps relatively short, periods in reproductive ontogeny when supplemental water is most beneficial for enhancing yield.

Although the yields of the late Clark isolines were comparable to that of

'Clark 63' (2136 kg/ha) grown in an adjacent variety trial (irrigation on July 13 and August 12 only), some indeterminate cultivars of similar maturity to Harosoy and early (e_2) Clark isolines yielded 3000-3300 kg/ha with lodging scores of 2.0-3.0. However, it seems reasonable to assume that the Dt_2 gene, when incorporated into possibly more complementary genetic backgrounds, could have some potential in reducing lodging in irrigated soybean culture and could well provide the necessary yield enhancement to allow soybeans to effectively compete with corn for grower-directed allocations of irrigated land resources.

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J. H. Williams

2) Heterosis and additive X additive epistasis.*

Abstract: A model is shown which has no dominance or dominance types of epistasis but which can result in heterosis of a hybrid over the best parent. The heterosis is due, in this case, entirely to additive X additive epistasis. The fact that dominance affects inbreeding depression but that additive X additive epistasis does not suggests that the amount of inbreeding depression is a better criterion for deciding whether or not to breed for hybrids in a particular crop.

Much has been said in recent years about heterosis in self-pollinated

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crops as a theoretical reason for going into hybrid breeding programs. I wish to show a model that contains no dominance whatsoever and still shows heterosis of the hybrid over the best parent. This is not a new idea but has been previously alluded to by Matzinger (1963), among others.

Assume a model with the following genetic values:

	bb	Bb	BB
aa	1	3	5
Aa	2	6	10
AA	3	9	15

This model contains only additive and additive X additive epistasis effects. If one crosses two lines, AAbb (value of 3), and aaBB (value of 5), one gets the genotype AaBb with a value of 6, thus exhibiting "hybrid vigor" or heterosis.

If one starts with the double heterozygote and self-pollinates to homozygosity without natural or artificial selection, one should end up with equal proportions of aabb, aaBB, AAbb, and AABB. The mean value of these four genotypes would be 6 and is equal to the value of the double heterozygote. Thus there is no inbreeding depression with this model. Therefore, the notions of "heterosis" and "inbreeding depression" should be considered separately. Inbreeding depression information should be better evidence for the presence or absence of large dominance effects or dominance types of epistasis. If no inbreeding effects are exhibited, one might conclude that the major genetic effects present are additive and additive X additive epistasis. This, in turn, would suggest the use of line per se recurrent selection programs as the best investment of breeding time and money, as suggested by Hanson et al. (1967).

One final point may be worth making. Suppose we change the model to the following genetic effects:

	bb	Bb	BB
aa	1	3	5
Aa	2	5	8
AA	3	7	11

Again, if we cross aaBB and AAbb the resulting hybrid would have the value of 5. One sometimes sees references to crosses being closer in measurement to one or the other of two parents, and this is sometimes called dominance.

The fact that this is not necessarily so is evident from this model, which again has only additive and additive X additive epistasis effects in it.

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1) Soybean breeding research in India.

Introduction: Soybean (Glycine max (L.) Merrill) is the miracle crop of the twentieth century. It is a new introduction to Indian agriculture. In view of chronic shortage of protein and oil in this country, soybean should be welcome introduction to provide the much needed stability and boost to the production of these two essential items of food (Saxena, 1975). Its high nutritive value makes it ideally suited for its versatile industrial uses. Its increasing industrial exploitation has, also, led to the manufacture of a large number of antibiotics in this country (Singh and Bajaj, 1969).

There is an immediate demand for soybean of 10,000 to 12,000 tons from the antibiotic industries of India, which utilize soybean as culture media. A demand for equal quantum is from the high protein food units. There is also a substantial demand from the poultry and animal feed industry (Jayswal, 1969).

Keeping in view the rising trend of demand for soybean from every corner of the country's economy, an overall improvement has to be undertaken in different agro-ecological conditions of India, to derive the fullest advantage from this wonder crop.

Floral biology: The knowledge of floral biology serves as a guideline to frame the various steps in proper execution of hybridization programs. The various aspects of floral biology, like bud development, time of blooming,

commencement and period of flowering, time of dehiscence of anther, viability of pollen grains and receptivity of stigma have been studied by Datta and Maiti (1966) and Lal et al. (1972).

Phenotypic and genotypic variability: Lal and Haque (1971) studied the phenotypic and genotypic variabilities in a collection of 36 varieties of soybean under rain-fed condition at the experimental farm of Ranchi Agricultural College, Kanke, in the rainy season of 1968. Heritability estimates (broad-sense) were found to be high for days to maturity, days to first flowering, period of flowering, 100-seed weight, number of leaves, number of nodes and total leaf area. They were moderate for the number of pods and low for seed yield.

Correlations among quantitative characters: Lal and Haque (1971) reported highly significant positive association of seed yield with number of leaves, total leaf area, plant height, number of nodes and number of pods. There was no significant association of seed yield with days to first flowering, days to maturity and 100-seed weight. The character 100-seed weight was negatively associated with almost all the characters studied except with period of flowering and seed yield where there was no significant correlation.

Kaw and Menon (1972) observed highly significant positive relationship of bean yield with number of beans, number of pods, days to 50% flowering, and plant height. Maturity also showed a positive association with yield.

Rohewal and Kopper (1973) reported that grain yield has positive correlation with number of branches, days to maturity, seeds per pod and 100-grain weight.

Path coefficient analysis: Lal and Haque (1971) indicated that total leaf area and plant height along with the direct yield attribute number of pods may be recommended as reliable selection indices.

Sengupta and Kataria (1971) reported that maximum weight should be given to days to maturity and leaves per plant which are directly related to the food manufacturing process of the plant.

Kaw and Menon (1972) observed that number of pods, days to maturity and days to 50% flowering are the three factors exerting the greatest influence both directly and indirectly upon the bean yield in space-planted tests under the short-day conditions of Coimbatore.

Rohewal and Kopper (1973) found that number of branches, number of grains per pod, 100-grain weight and days to maturity are more important.

Hence, for a plant breeder engaged in the improvement of soybean as regards yield, it will be necessary to lay maximum stress on these characters.

Objectives in breeding: The major breeding objectives have been high seed yield, maturity to fit the area of production, nonshattering pods, stronger stems, disease resistance and improved quality. Yield was the important consideration in all programs and chemical composition also received major consideration.

Color of seed is also an important consideration, yellow seed coats and yellow cotyledons being considered essential characteristics of a new variety.

In soybean, the pods generally burst open at maturity from both the sutures and shed seeds, resulting in considerable loss in grain yield. To overcome this difficulty, the strains reported to be nonshattering in other countries were introduced and tried under Delhi conditions. A Chinese variety, 'China Cluster', has proved to be comparatively nonshattering. It is a yellow-seeded determinate variety of medium maturity, producing pods in clusters. 'N49' from East Africa is another variety which is still more nonshattering. These varieties, however, have not proved to be high yielders. These could be utilized as useful breeding stocks in development of nonshattering varieties.

Breeding methods: Most of the existing varieties of soybean in India have been obtained through introduction.

Attempts to select suitable varieties of soybean have been continuing in various states such as Punjab, H.P., U.P., Maharastra and West Bengal for the last 25 years or so, but the selection work was based on a limited genetic material. The varieties so far cultivated in hilly areas were either small seeded, viny-types or bold seeded black and brown bushy types. In parts of M.P., a small black-seeded type locally called 'Kulth' has been popularly cultivated. A good yellow-seeded type has been selected about a decade back in Punjab and was distributed under the name 'Punjab-1' which has shown very wide adaptability.

As the soybean is a self-pollinated crop, and its flowers are very small, the success of breeding by hybridization is very limited. However, attempts are under progress by soybean breeders of India to evolve varieties by hybridization.

Choudhary (1972) reported that the 10 kr radiation treatment of gamma rays was effective in shifting the mean values in positive direction for plant

height, number of seed, 100-seed weight and seed yield in the variety 'Sepaya Black'.

Improved varieties for different agroclimatic zones: Germplasm from different parts of the world was systematically evaluated at several centers and the promising selections further tested in different parts of the country. Based on yield potential, reaction to diseases, seed quality and duration, varieties suitable for different agroclimatic zones of India have been identified (Singh and Saxena, 1975).

Northern Hill Zone: Bragg, 'Lee', 'Clark 63' and 'UPSM-19' are very promising. The last three are early maturing and take about 100-110 days. Bragg is a bit late, taking about 120 days to mature.

Northern Plain Zone: Bragg and Lee were found to be superior for northern plains and, therefore, they were released in 1969 for general cultivation. A new variety, 'Ankur' developed at Pant-Nagar was released for general cultivation in 1974. This matures in about 125-130 days.

Central Zone: Several varieties such as Ankur , 'UPSM-229', 'JS2', 'J231' and 'Davis', have been found suitable for this zone. JS2 and J231 are early, taking about 105 days. Ankur, UPSM-229 and Davis take between 110-120 days for maturity.

Southern Zone: Several varieties like 'Hardee', 'Improved Pelican', 'UPSS-69' and 'EC-39821', have been found to be promising.

Heterosis studies: Choudhary and Singh (1974) studied 17 F_1's involving eight promising soybean varieties to find out the extent and nature of heterosis. Hybrids Bragg X Clark 63 and Hardee X Punjab 1 exhibited maximum heterosis for seed yield, the values for which were 67.8 and 51.5% respectively.

Diallele analysis: Singh et al. (1974) conducted a diallele study for six quantitative characters in soybean at Punjab Agricultural University, Ludhiana, during Kharif 1972. The study revealed that complex characters, like grain yield, pods and clusters per plant, were controlled by additive and nonadditive gene action. The parent Bragg in general and the cross Bragg X 'Semmes' in particular were observed to be good and should be exploited.

Stability of varieties: Rohewal (1970) reported that the varieties Bragg and Lee showed their suitability for cultivation for high yielding environments and Improved Pelican and Punjab-1 for low yielding environments for the Northern and Central Plains. The ideal variety capable of being grown in all types of environment needs to be established.

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1) A high percentage of multifoliolate leaves in S.J. 2 soybean.

During the 1976 rainy season the multifoliolate plants (with four to five leaflets) were observed in an adapted and widely cultivated soybean cultivar, 'S.J. 2', grown at the Northeast Agricultural Center. A total of 3774 plants from every 10 rows was counted for this multifoliolate character. Plants with four or five leaflets were classed as multi-leafleted (Table 1). Multifoliolate plants constituted about 6.84%, which was a relatively high frequency in such a population with no previous selection. It was suspected that this genetical expression might have occurred, but unobserved, for years in this soybean cultivar.

Only plants with most leaves with five leaflets were tagged and harvested for further investigation. In December 1976, 130-plant rows were grown in the field for observation. A number of plant rows with multileaflets and some segregating rows were found, indicating that the multifoliolate character is controlled by a single major gene. This gene may be the same as that reported by Fehr (1972), $\underline{Lf}_1$. Further investigation is underway.

Table 1
Percentage of plants in S.J. 2 soybean cultivar
showing multifoliolate character

	Row									
	1	2	3	4	5	6	7	8	9	Total
Plants counted	310	347	326	475	233	443	479	531	630	3774
Plants with multileaflets	21	33	26	27	16	47	31	26	31	258
Multileafleted plants, %	6.8	9.5	8.0	5.7	6.9	10.6	6.5	4.9	4.9	6.84

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1) Selecting homozygous male fertile lines from an intermating soybean population.

The synthesis of random-mating soybean populations and their use in recurrent selection programs was enhanced by the discovery of the male sterile genotype, $\underline{ms}_1$ $\underline{ms}_1$ (Brim and Young, 1971; Brim and Stuber, 1973). At some point in such a recurrent selection program, a plant breeder usually wishes to select lines for testing from one or more of the improved populations. There is a difficulty in this, however, because the progeny of every fertile plant selected from a random mating population will segregate for male sterility. This is because every fertile plant is an F_1 from a cross between a male sterile and a fertile plant and is therefore heterozygous at the $\underline{ms}_1$ locus. The following describes a simple technique for deriving homozygous fertile lines from a random mating population using single-seed descent.

Begin by selecting $\underline{x}$ F_1 fertile plants from the random mating population. Grow the progeny of each plant in a row. This results in $\underline{x}$ rows, each of which represents a different F_2 family. A set of lines are derived from the F_2 families by sampling a single seed from $\underline{y}$ plants in each row. This results in a total of $\underline{x} \cdot \underline{y}$ lines which can be inbred by single-seed descent. In each generation of inbreeding, sample only the fertile plants for the next generation of inbreeding. At some generation, F_n, the fertile plants are harvested and each increased in plant rows (the F_{n+1} generation). In the F_{n+1} generation, only the fertile lines which do not segregate for sterility are saved. The result will be a group of F_n-derived lines which are homozygous for the dominant $\underline{Ms}_1$ allele.

Table 1 shows the proportion of fertile plants which can be expected with each generation of selfing, assuming the steriles are discarded each time. It also shows the number of F_n-derived $\underline{Ms}_1$ $\underline{Ms}_1$ lines which one can expect at the n^{th} generation. The number of F_n-derived lines depends upon $\underline{x} \cdot \underline{y}$, the original number of plants sampled. It should be noted that the proportion of F_n-derived lines increases by one-half of the proportional increase in the F_{n-1} generation.

Table 1

Proportions of homozygous male fertile lines which can be expected with each generation of inbreeding by single seed descent. Lines selected from a random mating soybean population

Generation	Proportion of F_n fertile plants	Expected number of F_n derived $\underline{Ms}_1\underline{Ms}_1$ lines	Proportional increase in expected $\underline{Ms}_1\underline{Ms}_1$ lines
F_2	3/4	.500 x · y[†]	
F_3	5/6	.583 x · y	.083
F_4	9/10	.625 x · y	.042
F_5	17/18	.646 x · y	.021
F_6	33/34	.656 x · y	.010
F_7	65/66	.661 x · y	.005
⋮	⋮	⋮	
F_∞	1	.666 x · y	

[†] x and y represent the number of F_2-plant rows and the number of plants sampled within each row, respectively.

The table can be used to decide how many F_2 plants should be sampled in order to have a specified number of F_n-derived lines for testing. For example, if 500 F_4-derived lines were needed, it would be necessary to begin with a population of $\underline{x} \cdot \underline{y} = 800$, since .625 x 800 = 500. The relative sizes of $\underline{x}$ and $\underline{y}$ would be an individual judgment based on the size of the base random mating population and the number of generations it has intermated. The results in Table 1 can also be generalized to any recessive simply-inherited trait that can be easily identified and selected out of a population with each generation of selfing.

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1) Techniques developed for screening soybeans for Mexican bean beetle resistance.

The lack of uniform infestations of Mexican bean beetle (Epilachna varivestis Mulsant) in field plantings can make selection for resistance in segregating soybean populations very difficult. For this reason, the results from screening F_2 plants in the field have been inconsistent. An alternative is to advance F_2 plants to the F_3 to provide replication at more than one location. However, this procedure is costly in both time and land requirements. The development of supplemental greenhouse screening techniques which provide precise, repeatable results is essential to the progress of the breeding program. We report here a comparison of two such techniques, adult beetle feeding on mature soybean trifoliates and on unifoliate soybean seedlings, to foliar feeding in field nurseries.

Field Evaluations: In 1976, F_3 progenies from a backcross to a susceptible adapted parent were planted in randomized complete block designs with two replications/entry. Rows were 4.8 m long, spaced 1.1 m apart; and each row contained 25 seed. Every third row in each nursery consisted of Henderson bush lima beans, and 'Davis', 'Clark', and 'Forrest' soybeans in a 1:1:1:1 mixture, planted 14-21 days before test lines, to increase and hold incoming beetle populations in the nurseries. Populations were monitored weekly, and when extensive defoliation was evident, a visual estimate of defoliation from 0 to 60% in 5% increments was recorded.

Greenhouse Evaluations:

Excised trifoliate tests: The 9th and 10th trifoliates from field grown F_3 progenies were placed in water-filled 250 ml Erlenmeyer flasks and placed in a randomized complete block design (10 replications/treatment) on the floor of a 120 x 120 x 90 cm wooden frame cage covered with Saran screen. The cage was then infested with beetles (1/trifoliate), placed on a bench in a shaded, open-air insectary, and covered with a double layer of black floral cloth to lessen the interference of sunlight. After 48 hr, feeding damage was visually estimated on a scale of 1-12: 1 = extensive feeding in 1 of 12 trifoliate

quadrants; 12 = extensive feeding in all of the 12 trifoliate quadrants, and converted to percent defoliation.

Seedling tests: Seed from each of the test progenies and the resistant and susceptible checks were planted in randomly arranged rows in wooden flats containing a sand, soil, and peat potting mix. When unifoliate leaves were present on all seedlings, cotyledons were clipped off, and the plants were infested with beetles (1/seedling). Small wooden frame cages with Saran screen tops and foam rubber strips along the bottom edge were then placed over the flats. After 72 hr, seedlings were rated for damage on a scale of 1-8: 1 = extensive feeding in 1 quadrant of the 8 quadrants in the two unifoliates; 8 = extensive feeding in all 8 quadrants of the two unifoliates. In both seedling and excised trifoliate tests, 2-wk-old female beetles were used for testing after the following pretest conditioning: 24 hr feeding on Forrest leaves; 24 hr with access to distilled H_2O.

Of the lines evaluated in the field, 13 were found to have moderate to high levels of resistance. For purposes of illustration, the responses of 4 of these are shown in Table 1.

Table 1
Adult Mexican bean beetle feeding on selected progeny in three types of evaluations

Line	Field	Greenhouse	
	$\bar{x}$ % Defoliation[1]	Excised trifoliates $\bar{x}$ % defoliation	Seedling $\bar{x}$ damage[2]
Resistant check	5 a[3]	8 ab	0.8 a
1	7 a	14 abc	0.4 a
2	7 a	15 abc	0.9 a
3	10 ab	26 c	2.0 a
4	14 ab	24 bc	2.8 a
Susceptible check	30 c	44 d	5.5 b

[1]Mean damage computed for data at three locations.

[2]1 = no damage; 8 = heavy feeding on both unifoliate leaves.

[3]Means in each column not followed by the same letter differ significantly at the .05 probability level as determined by Duncan's Multiple Range Test.

Drought conditions may have affected the uniformity of beetle infestation in the field, thus tests with excised trifoliates were conducted to more critically evaluate the resistant lines. Results of these tests were similar to those of field evaluations, but lines 3 and 4 proved to be more susceptible (Table 1).

Similar results were also obtained in feeding tests with seedlings of the same 13 lines and these results and those of field evaluations were closely correlated ($r = .63^{**}$, 12 df). Seedling tests did not detect susceptible lines as accurately as did the excised trifoliate test, but ratings of the more resistant lines were similar in both tests. Additionally, results of both tests were also highly correlated ($r = .71^{**}$, 11 df). The results of these tests indicate the usefulness of each to a breeding program. The utility of excised trifoliate and/or seedling screening tests of F_2 plants in the greenhouse is yet to be determined. These tests would greatly increase the efficiency of the breeding program. Both techniques are now being used to increase the efficiency and precision of insect resistance evaluations.

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1) Characterization of cytoplasmic diversity in soybeans.

Soybean cultivars which occupy a majority of the U.S. acreage trace to only a few maternal parents. According to a recent report (1972) by the National Academy of Sciences, the maternal ancestors and their combined frequency of occurrence in the parentage among Northern and Southern varieties are: 'Mandarin' 51%, 'Illini' 23%, 'Tokyo' 11%, 'Dunfield' 8%, 'Mukden' 4%, and 'Roanoke' 4%. Four of these parents are introductions from Manchuria, one from Japan, and one is of unknown origin. The limited geographical origin and the paucity of maternal parents is a reasonable basis for concluding that a high degree of cytoplasmic uniformity exists in currently grown soybean

cultivars. The consequences of cytoplasmic uniformity in maize suggest that plant breeders should give consideration to choosing parents diverse in cytoplasmic constitution. We report here preliminary results from a study designed to assess cytoplasmic diversity in soybeans.

Because classical genetic analyses are not applicable to a study of cytoplasmic traits in soybeans, a restriction endonuclease fragment analysis (Nathans and Smith, 1975) was used. This approach is contingent on the presence of specific sequence differences among the organelle DNAs. Levings and Pring (1976) recently described the application of this technique in characterizing mitochondrial DNA in maize. The method consists of an analysis of the DNA fragments generated from digestion of organelle DNA by a site-specific restriction endonuclease. The resulting fragments are separated by gel electrophoresis, producing a "fingerprint" of the original DNA molecule. The fingerprints of organelle DNAs from test genotypes can then be compared. In this study, mitochondrial DNAs (mtDNA) isolated from the hypocotyls were digested with the restriction endonuclease Bam 1 from Bacilis amyloliquifaciens H.

In addition to the six maternal ancestors noted above, 'Arksoy', 'Dorman', Glycine soja and G. gracilis were chosen for study. The variety 'Lincoln', from the cross of Mandarin X Manchu, was substituted for Mandarin due to problems with seed availability. Arksoy, introduced from Korea, was chosen as a representative of an additional geographic location. Dorman, from the cross Dunfield X Arksoy, has been used as a parent in a number of improved varieties. Glycine soja and G. gracilis represent the supposed progenitor, and an intermediate species between the progenitor and G. max, respectively.

Electrophoresis of the restricted mtDNA fragments resulted in approximately 40 bands. Banding patterns of all six maternal ancestors were identical. Figure 1 is a schematic representation summarizing the differences in electrophoretic banding patterns that were observed among the mtDNAs examined. Only the top 5 cm of the gel is shown since no differences were detected in the remainder of the gel. The center banding pattern represents a fingerprint of mtDNA from Dorman and is identical to the fingerprints obtained for all the maternal ancestors as well as for G. gracilis. Arksoy, the variety of Korean origin, differs from Dorman by a single missing band at approximately 3 cm. The wild species, G. soja, differs by six bands, all occurring in the upper 4 cm. In general, there was a high degree of homology among the banding patterns of the mtDNAs examined. The heterogeneity in banding patterns reflects

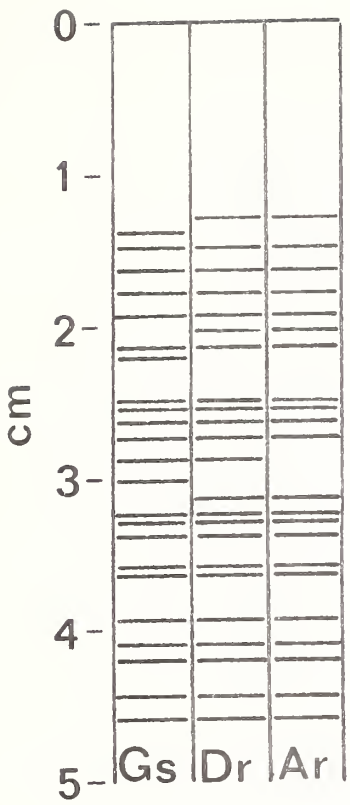

Figure 1. Schematic diagram illustrating the different banding patterns obtained by gel electrophoresis of soybean mtDNA digested with restriction endonuclease Bam 1. Sources of mtDNA were: (Gs) Glycine soja, (Dr) Dorman, and (Ar) Arksoy. Only upper 5 cm of the 10 cm gels is shown.

differences in the number and position of cleavage sites only and are not necessarily related to specific fitness traits. Nevertheless, these results clearly demonstrate a high degree of uniformity among mtDNAs of the maternal ancestors when digested by Bam 1. This conclusion is reinforced by the differences in banding patterns observed for G. soja and Arksoy compared with the maternal ancestors. These results, then, tend to substantiate the premise that cytoplasmic uniformity cannot be overlooked as a potential hazard to production in soybeans. Of further interest is that restriction endonuclease analysis provides a powerful tool for soybean breeders in detecting maternal parents of diverse cytoplasmic constitution for use in varietal development.

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1) Identification of male sterile soybean plants by pollen examination.

The male sterile maintainer line, N69-2774, segregates 1:1 for fertility and sterility (Brim and Young, 1972). The fertile and sterile siblings are indistinguishable, except for pollen grain morphology, until the onset of maturity. For controlled hybridization the phenotypic similarity is an obstacle. To avoid contamination, male fertile plants of the maintainer must be removed from crossing blocks as quickly as possible. Pollen grains of the male sterile plants are typically larger than those of fertile siblings, appear to be fewer in number, do not germinate or disperse in a germination medium, but remain in clumps similar to the crescent shape of the anther (Brim and Young, 1971). We describe here a precise and rapid technique of pollen examination to distinguish between male fertile and male sterile plants in a segregating population.

Flowers collected from individual plants in the population are desiccated over calcium chloride prior to examination. One hour of desiccation is sufficient for anther dehiscence unless relative humidity is unusually high. Two or more flowers are usually collected from each plant to reduce the risk of choosing flowers which will not dehisce. Because pollen sterility of the male sterile plants is complete, a single flower is enough to classify the plant. It should be noted that at Raleigh, NC it is more difficult to obtain suitable flowers for classification from greenhouse-grown plants. No such difficulties have been experienced with field-grown plants.

A hanging-drop slide of the type available from most glassware supply houses is used in classification. A 30% sucrose solution constitutes the germination medium. A single drop of the medium is placed on a 22 X 22 mm cover slip. The flower is emasculated to expose the anthers and pollen is dispersed in the media by gently tapping the flower above the drop. The rim of the hanging-drop slide is lubricated with vaseline which serves as an adhesive. The slide is then placed over the drop on the cover slip and quickly inverted.

The pollen grains in the drop are examined under a binocular microscope at 10X magnification. Pollen tube formation of fertile pollen grains is usually apparent in 15-20 minutes at room temperature (75-80°F). Once the flowers are collected one individual can classify approximately 50 slides per hour.

Pollen grains from male sterile plants are readily distinguished in the hanging drop. Occasionally, shrunken, well-dispersed pollen grains which do not germinate are detected. These are usually from anthers which have dehisced the day prior to sampling and upon resampling prove to be from fertile plants.

This method provides a technique for removing fertile plants from the maintainer line in order to achieve controlled natural hybridization. Because the maintainer line has white flowers and grey pubescence, pollen donors carrying dominant markers can be used as parents in natural crosses thus avoiding the need to identify fertile plants in the maintainer. But where appropriate pollen donors are unavailable for the experimenters' purposes, then classification as outlined above is necessary.

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1) Induction of sterility in soybeans with ethidium bromide.

Ethidium bromide (EB), an acridine compound which binds to nucleic acids, has been used as a mutagen in peanuts by Levy and Ashri (1975). Burton and Hanna (1976) induced cytoplasmic male sterile mutations in pearl millet with EB and suggested that EB as a cytoplasmic mutagen might aid in the development of a cytoplasmic male sterile nuclear restorer system in other crops. The following is a report on preliminary attempts to induce cytoplasmic male

sterility in soybeans using seed treatments of ethidium bromide.

Before treatment, soybean seeds were wrapped in damp paper towels for 16 hr (overnight). This was necessary to prevent seeds from rupturing when introduced to the EB solutions. The seeds were then soaked in distilled water solutions of EB at room temperature and in the dark. (EB is inactivated by light.) At the end of the treatment period, the seeds were rinsed, wrapped in wet paper toweling and placed in a 28°C incubator for two days to test for germinability.

In the beginning, we found that the treatment concentrations used by Levy and Ashri for peanuts (500 ppm EB for 24 to 168 hr) were too severe for soybeans. After several trials, we found soybeans to be more tolerant to treatments in the range from 500-62.5 ppm EB for 1 to 4 hr and decided to use that range for more extensive testing.

In January 1976, we planted, in pots in a greenhouse, 'Lee' and 'Bragg' seeds which had been treated with EB concentrations of 150 ppm for 1½ hr. The time was increased to 3 hr for Bragg because it seemed unaffected by the 1½ hr treatment. Among the plants which survived, there were many which showed varying degrees of stunted growth. Some of the plants showed partial or complete sterility.

In the spring 1976, seeds of the varieties 'Lee 74', 'Ransom' and 'Jackson' were treated with solutions of 250 ppm EB for 3½ hr and planted immediately in the field. We found that the stunted phenotypes which we observed in the greenhouse did not survive in the field. Table 1 shows the number of seeds treated, the number of plants which survived to maturity, and the number of plants recovered which appeared to be sterile or partially sterile. These plants had one or more of the following characteristics: rosettes of undeveloped pods at some of the nodes, reduced seed set, and a preponderance of one-seeded pods.

The seeds produced on the sterile-appearing plants or plant parts were presumably the result of natural cross-pollination. These seeds will be planted in the field in the spring 1977. If the sterility is cytoplasmically inherited, it should be evident among the progeny provided that a dominant fertility restorer gene was not contributed by the pollen parent.

The low survival rate of the treated seeds in the field, indicated the need for lower treatment concentrations. Seeds of Ransom, Jackson and Lee 74 were further tested with concentrations of 500, 250, 125, 62.5 and 0 ppm EB

Table 1

Field survival rate and the number of sterile plants produced by ethidium bromide treatment

Variety	No. of seeds treated	No. of plants surviving to maturity	No. of sterile-appearing plants
Ransom	8,000	245	7
Jackson	3,500	193	4
Lee 74	8,000	15	1

for 2½ hr. After treatment, the seeds were germinated for 2 days, then planted in sand flats and grown for 3 weeks. The seedling survival rates are presented in Table 2. There was, in general, a linear increase in survival as the concentration of EB decreased. It should be noted that the percent survival under field conditions would probably be less than the survival rates shown in Table 2.

Table 2

Mean percent survival of soybean seedlings grown for 3 weeks in sand flats

Treatment concentrations (ppm) of EB	Variety		
	Lee 74†	Ransom†	Jackson††
500	18.6	31.0	50.0
250	39.6	53.0	63.0
125	49.4	73.0	80.5
62.5	71.0	82.6	82.5
0	84.0	83.4	92.0
	lsd = 15.4	lsd = 11.6	lsd = 11.2
	cv = 24.2	cv = 15.0	cv = 9.8

†means of 6 replications, 50 seeds per replication.

††means of 4 replications, 50 seeds per replication.

In conclusion, our preliminary results show that EB will induce sterility in soybeans. It also appears that there are definite genotypic differences in sensitivity to EB.

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Table 1

Recent additions to the Soybean Genetic Type Collection List[†]

Strain	Genes or description	Source	Maturity	Code
T263	dwarf	Found in Harosoy 63 X PI 257.435 in 1968 in the Iowa State University nursery in Hawaii. A74-2	II	PGNBr DYY
T264	dense pubescence	Found in neutron-irradiated 'Blackhawk' in the N_2 generation at Urbana in 1956. L58-2749	I	WGNBr SYBF
T265H	chlorophyll deficient	Found in Williams[6] X T259 in the F_2 generation in 1974-75 in the greenhouse at Urbana. L75-0324	III	WTNTn SYBl
T266H	$\underline{ms}_1$-Urbana	Found in an F_3 row of L67-533 (Clark-S) X SRF300 at Urbana in 1971. See Soybean Genet. Newsl. 2: 49-51. 1975. [higher female fertility than T260]	IV	TTNBr -YBl
T267H	$\underline{ms}_1$-Tonica	Semi-sterile plant found in a field of Harosoy by F. M. Burgess, Tonica, IL, in 1955. L56-292	II	PGNBr DYY
T268H	$\underline{ms}_1$-Ames, $\underline{St}_4$	Semi-sterile plant found in T258 at Ames, IA, in 1970. A73g-21	II	PGNBr DYY

[†]For additional information see Soybean Genetics Newsletter 3: 62-67. 1976.

Table 2

Genetic linkage groups in soybeans

Linkage group	Linkage intensity map[†]
Linkage Group 1	
dwarf (T263)	y_{12} 20.2(1.1) e_1 3.9(0.4) t
e_1 early maturity	dwarf 15.4(1.0) t
fg_3 flavonol glycoside	fg_3 13.7(6.6) t
fg_4 flavonol glycoside	fg_4 0 t
t gray pubescence	fg_4 12.0(1.8) fg_3
y_{12} chlorophyll deficient	
Linkage Group 2	
p_1 nonglabrous plant	p_1 20.9(2.4) r
r brown seed	
Linkage Group 3	
d_1 green seed embryo	g 4.2(0.6) d_1
g yellow seed coat	
Linkage Group 4	
ln narrow leaf	v_1 35.6(0.9) ln 26.4(1.4) p_2
p_2 puberulent plant	
v_1 variegated leaf	
Linkage Group 5	
dt_1 determinate stem	dt_1 39.4(1.8) l_1
fg_1 flavonol glycoside	dt_1 39.8(3.0) fg_1
l_1 tan or brown pod	
Linkage Group 6	
df_2 dwarf plant	df_2 12.1(0.7) y_{11}
y_{11} chlorophyll deficient	
Linkage Group 7	
i self dark seed coat	y_{13} 31.3(1.9) o 17.8(0.7) i
o red brown seed coat	rhg_4 ? i
rhg_4 susceptible to cyst nematode	
y_{13} chlorophyll deficient	
Linkage Group 8	
ms_1 male sterile	w_1 29.7(1.6) ms_1
w_1 white flower	w_1 2.2(0.5) wm
wm magenta flower	

[†]Linkage intensity map values given as percentage recombination, standard errors enclosed in parentheses: % R (SE).

Table 3

F_2 linkage data for new linkage information in soybeans[†]

Genes		Phenotypic classes								Cross	Reference
a	b	AB	Ab	aB	ab	Sum	% R	SE	Phase	No.[††]	No.[††]
T263 (dwarf)	t	1158	130	112	300	1700	15.4	1.0	C	2	5
dt_1	fg_1	135	33	31	16	215	39.8	3.0	C	1	4
fg_1	dt_1	135	31	33	16	215	39.8	3.0	C	1	4
fg_3	fg_4	247	19	20	71	357	12.0	1.8	C	3	1
fg_3	t	111	48	59	1	219	13.7	6.6	R	4	4
fg_4	fg_3	247	20	19	71	357	12	1.8	C	3	1
fg_4	t	111	47	63	0	221	0	0.0	R	5	1
ms_1	w_1	1268	583	557	60	2468	30.4	1.8	R	6	5
ms_1	w_1	451	87	91	100	729	27.9	2.0	C	7	5
t	T263 (dwarf)	1158	112	130	300	1700	15.4	1.0	C	2	5
t	fg_3	111	59	48	1	219	13.7	6.6	R	4	4
t	fg_4	111	63	47	0	221	0	0.0	R	5	1
w_1	ms_1	1268	557	583	60	2468	30.4	1.8	R	6	5
w_1	ms_1	451	91	87	100	729	27.9	2.0	C	7	5
w_1	wm	333	6	4	107	450	2.2	0.5	C	8	3
w_1	wm	778	379	387	0	1544	0		R	9	2[†††]
wm	w_1	333	4	6	107	450	2.2	0.5	C	8	3
wm	w_1	778	387	379	0	1544	0		R	9	2[†††]

[†]Data for Linkage Groups 1-7 not included; see references for Linkage Groups 1-7.

[††]See Tables 4 and 5 for crosses and references, respectively.

[†††]See also Buzzell, R. I., R. L. Bernard and B. R. Buttery. 1974. Soybean Genet. Newsl. 1: 14-15.

Table 4

Cross No.	Crosses	Reference
1	OX250 (fg_1 dt_1) X OX922 (Fg_1 Dt_1)	4
2	T263 (dwarf t) X disomics and trisomics A and B (tall T)	5
3	T31 (Fg_3 Fg_4) X OX936 (fg_3 fg_4)	1
4	Blackhawk (Fg_3 t) X Kingwa (fg_3 T)	4
5	AK-FC 30.761 (fg_4 T_1) X Beeson (Fg_4 t_1)	1
6	T266 (ms_1 W_1) X disomics and trisomics A and B (Ms_1 w_1)	5
7	T260 (ms_1 w_1) X disomic and trisomic C (Ms_1 W_1)	5
8	OX281 (w_1 wm) X Beeson (W_1 Wm)	3
9	L62-904 (w_1 Wm) X T235 (W_1 wm)	2

Table 5

Reference	References for new linkage information
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3	Buzzell, R. I. 1976. Soybean Genet. Newsl. 3: 11-14.
4	Buzzell, R. I. 1977. Soybean Genet. Newsl. 4: 12-13.
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